TREATMENT OF HIGH NITROGENOUS WASTES BY FLOCCULATING ALGAL-BACTERIAL SYSTEM

A Thesis Submitted
in Partial Fulfilment of the Requirements
for the Degree of
DOCTOR OF PHILOSOPHY

By
K. RAGHAVAN NAMBIAR

to the
DEPARTMENT OF CIVIL ENGINEERING
INDIAN INSTITUTE OF TECHNOLOGY, KANPUR
DECEMBER 1978

In Loving Memory of My Parents

PUTHANVEETIL KELU NAMBIAR

and

KEEZHANDUR LAKSHMI AMMA

CERTIFICATE

This is to certify that the thesis "Treatment of High Nitrogenous Wastes by Flocculating Algal-Bacterial System" submitted by Shri K. Raghavan Nambiar, in partial fulfilment of the requirements for the Degree of Doctor of Philosophy at the Indian Institute of Technology, Kanpur is a record of bonafide research work carried out under my supervision and guidance. The work embodied in this thesis has not been submitted elsewhere for a degree.

S.D. Bokil

(S.D. BOKIL)
Assistant Professor
Department of Civil Engineering
Indian Institute of Technology
Kanpur

Dated: December 8, 1978

ACKNOWLEDGEMENT

It is with extreme respect and warm feelings, that the author wishes to express his deep sense of gratitude to Dr. Shyam D. Bokil for guiding and encouraging the author throughout this research programme. But for his far-sightedness and encouragements, this thesis would not have taken this shape. The author wishes to express his sincere thanks to.

— Dr. A.V.S. Prabhakara Rao for his sincere interest and helpful suggestions

— Dr. G.D. Agarwal for his kind advices and suggestions during the early part of this research

— Dr. Malay Chaudhuri for his kind helps and encouragements during the study

— Dr. C. Venkobachar for his valuable suggestions and advice

— Dr. Leela Iyengar for her kind helps during experiments and literature survey.

The author expresses his heart-felt thanks to Mr. R.C. Adhikari for his unqualified helps during this research. His thanks are due to Messers A. Hassan, N.R. Sahu, S.N. Mishra and Ram Singh for their timely helps during laboratory works.

The author wishes to thank Messers Balagopal, Vinod Tare, Eshwar, Vishveshwara Rao, Sheldarkar and Madhu Sharma for their kind co-operation in the compilation of the thesis.

The author expresses his high appreciation and thanks for the marvellous typing work done by Mr. R.N. Srivastava.

The author is highly thankful to the Government of Kerala; Dr. S. Vasudev, Director of Technical Education; Dr. P.J. George, Principal and Professor P.U. Paul of the Government Engineering College, Trichur for their kind helps and encouragements for deputing him for Ph.D. programme.

Last, but not the least, the author places on record his indebtedness and great sense of gratitude for the inspiration, encouragement and unbounded enthusiasm imparted to him through letters, by his beloved wife, Mrs. Sushecla Nambiar and his daughters, Vijya and Vidhya.

K. Raghavan Nambiar

TABLE OF CONTENTS

Chapter Page

Chapter | Page

Chapter Page

LIST OF TABLES

LIST OF FIGURES

SYNOPSIS

K. RAGHAVAN NAMBIAR
Ph.D.
Indian Institute of Technology Kanpur
December 1978

TREATMENT OF HIGH NITROGENOUS WASTES BY FLOCCULATING ALGAL-BACTERIAL SYSTEM

Concomitant with the emergence of a large number of giant fertiliser plants besides the existing ones, the adverse effects of high nitrogenous wastes on water bodies have assumed colossal dimensions. Eutrophication, toxicity to fish and other aquatic life, exertion of high oxygen and chlorine demand are some of the deleterious effects inflicted by high nitrogenous wastes

Few biological and physico-chemical methods are available for the effective treatment of high nitrogenous wastes. Air-stripping, biochemical nitrification-denitrification, simple lagooning and algal ponds are some of the popular methods available for the treatment of high nitrogenous wastes. Ammonia removed by air-stripping method is bound to come back to the water bodies during rains. Moreover the method is an expensive one. Operational troubles is another negative point in this process.

Biochemical nitrification-denitrification is an efficient process. However nitrification is adversely affected by the

presence of large amount of ammonia. Moreover the efficiency of nitrification is not very high. Considerable amount of untreated ammonia escapes into the denitrification units. Moreover organic carbon such as methanol is necessary for effective denitrification. After denitrification the gaseous nitrogen products are released into the atmosphere, thereby rendering the ammonia to no useful purpose.

Simple lagooning and algal ponds are no doubt cheaper. However they require very large areas and their efficiencies are also very limited. Very large quantity of biomass escaping along the effluent is a drawback of these processes. In all the above methods ammonia alone is tackled and urea is left untouched.

It is from this back drop that flocculating algal-bacterial system was tried as a method for treating high nitrogenous wastes. Urea as well as ammonia are effectively removed from the wastes in the flocculating algal-bacterial system.

The research was divided into three phases of study. Phase 1 essentially consisted of batch studies. Parameters such as biomass concentration, algae/bacteria ratio, detention time, total nitrogen and COD removal efficiencies were involved in the studies. Organic carbon was fed into the reactors besides ammonia, urea and phosphates. Tapwater was used to make up the volume. C:N:P ratios ranging from 30:10:1 to 3:10:1 were tried in various batch studies. Stoichiometric

C:N:P ratio was determined to be 30:10:1. Sewage was tried as a substitute of organic carbon. One percent carbon dioxide in the air also was tried as a supplement of inorganic carbon. The feed contained 1000 mg/l of nitrogen. Effect of pH on total nitrogen removal efficiency was also studied. The reactor contents were aerated at 1 lpm/l in sunlight.

Phase 2 studies involved the determination of optimum conditions for the maximum nitrogen removal efficiency. C:N:P ratio of the feed was 10:10:1. This ratio was found to be optimum for efficienct performance of single reactor in batch studies in Phase 1. Three batch reactors were run in series in this Phase. Optimum detention time of 2 days as obtained in Phase 1 was adopted in the studies. Optimum biomass concentration in each of the reactors was found out with respect to maximum total nitrogen and COD removal efficiencies. The effluent of Reactor 1 was influent of Reactor 2 and the effluent of Reactor 2 served as influent of Reactor 3. Organic carbon was added to the effluents of Reactors 1 and 2 so as to make up the C:N:P ratio 10:10:1. pH in one set was maintained at 8.0-8.3.

Phase 3 studies involved in operating a continuous flow single reactor to which a feed containing 1000 mg/l of nitrogen (ammonia nitrogen 700 mg/l and urea nitrogen 300 mg/l) at a C:N:P ratio of 10:10:1 was continuously fed. The liquid detention time provided was 1 day. The reactor was

operated at solid retention times of 10, 8, 6 and 4 days. At each solid retention time determinations of ammonia nitrogen, urea nitrogen, nitrite nitrogen, nitrate nitrogen, COD, pH, alkalinity, biomass concentration and algal concentration were carried out as per methods given in the thesis. The determinations were done only after the steady state conditions were attained.

From these observations the biokinetic constants such as the yield coefficient, Y, the microorganism decay rate, k_d, the maximum specific growth rate, $\bar{\mu}$, and the saturation constant defined as substrate concentration at which $\mu = \frac{\bar{\mu}}{2}$, K_s were determined. Revised stoichiometric equations were developed for different solids retention time.

An optimum detention time of 2 days would remove as much as 75 percent of total nitrogen at a C:N:P of 30:10:1. However at a C:N:P ratio of 10:10:1 when urea and ammonia were present at a concentration of 1000 mg/l nitrogen the efficiency at 2 days detention time was 56 percent. Temperature had conspicuous effect on the system. Higher the C:N:P ratio the higher the total nitrogen removal efficiency.

Carbon dioxide had little effect on improving the total nitrogen removal efficiency. Sewage would not be suitable as a source of organic carbon in the treatment of high nitrogen wastes. Among the four methods tried, namely, activated sludge system, algal system, flocculating

algal-bacterial system and simple aeration under identical conditions and feed C:N:P ratio of 10:10:1 and total nitrogen concentration of 1000 mg/l, flocculating algal-bacterial system was found to be the best from the point of view of biomass stability, effluent clarity, total nitrogen, urea and COD removal efficiencies. pH in a range of 8.0-8.3 was having a favourable effect for rapid and enhanced total nitrogen removal efficiency.

It was also observed that luxury uptake of nitrogen by the biomass exists in the flocculating algal-bacterial system treating high nitrogenous wastes at pH 8.0-8.3. The sludge nitrogen concentration was found to be 9-16 percent of the biomass. Higher percentage was incorporated at detention times of 4-6 days under carbon stressed conditions. This is backed by theoretical explanation of the free flow of ammonia into the cells at pH 8.0-8.3.

Compared to a single batch reactor, studies here have indicated that about 3 batch reactors in series give much better total nitrogen removal efficiency under identical conditions.

Biokinetic constants were evaluated in Phase 3 study. The total nitrogen concentration in the feed was 1000 mg/l at C:N:P ratio of 10:10:1. pH was maintained at 8.0-8.3. The optimum parameters were found to be as follows:

Liquid detention time (days)	= 1
Solids retention time (days)	= 8-10
Maximum efficiency of total nitrogen removal (percent)	= 70
Maximum specific growth rate (day^{-1})	= 0.5
Microorganism decay rate (day^{-1})	= 0.01
Yield coefficient (mg biomass/mg total N removed)	= 8

Since glucose as a source of organic carbon supplement is expensive, efforts were made to replace it by other high carbon industrial wastes. The waste product of dairy industry, namely, whey was tried as a source of organic carbon in a set of batch experiment. The response was quite favourable and experiment successful. The total nitrogen and COD removal efficiencies as well as the settleability of the resulting sludge were quite comparable to those in which glucose was used. So flocculating algal-bacterial system offers an elegant, cheap and successful method for treating urea and ammonia present in high nitrogenous wastes.

1. INTRODUCTION

1.1. General:

In the long history of man's evolution, population has been dependent upon the availability of food. In the quest of producing more and more of food to meet the increasing demand man began to shape the biosphere to his own ends. The continuing expansion of land under the plough and evolution of chemically oriented agriculture are producing ominous changes in the biosphere on a global scale. As a result natural cycles of energy and chemical elements are obviously getting affected to a considerable extent.

1.2. Impact of New Technology on Nature's Balance:

When the availability of land became scarce, man invented techniques to raise productivity of land already under

cultivation. These techniques tend to alter the balance between biosphere, lithosphere, hydrosphere and atmosphere. Modern agriculture depends mainly on four technologies, namely, mechanisation, irrigation, fertilisation and chemical control of weeds and pests. Each of the above technologies contributed its own share in affecting the environment in one way or other. Consequently natural cycles of energy and elements and ecosystems are put to disasterously drifting pathways.

Chemical fertilisers were abundantly introduced only recently for increasing productivity. However, the highly concentrated wastes generated have a great pollution potential. The treatment of these wastes is currently one of the most important problems facing the developing countries. The principal ingredients of chemical fertiliser are nitrogen, phosphorus and potassium. The run off of chemical fertiliser from producing centres and agricultural lands into rivers, lakes and underground waters creates two important hazards. One is the chemical pollution of drinking water. The second one, which is much more extensive in its nature is the well-known phenomenon called eutrophication. Inorganic nitrates and phosphates discharged into lakes and other bodies of fresh water provide rich medium for the growth of algae. In the end the eutrophication of the lake brings about its death as a body of freshwater, converting it into a swamp.

1.3. Water Pollution – A Pressing Problem:

World's water exists as liquid, solid and as vapour. It had been reported by Penman (1970) that the total volume is of the order of 1500 million cubic kilometres. Oceans and seas make up about 97 percent of all water. Of the remaining 3 percent three quarters is locked up as solid in the polar ice caps and in glaciers. The balance is constituted by underground water, surface water and water vapour. The surface water works up to only 2 percent of the underground water while water vapour is only a very small fraction of the same. It is this very small fraction of surface and atmospheric water that sustain the balance of nature. It is clear, how important, it is to protect the hydrosphere and atmosphere, while man is putting his greedy hands on the biosphere for his own ends.

In India there is a growing awareness of meeting the supply of food indigenously by adopting new technologies of mechanisation, irrigation, fertilisation and chemical control of pests and weeds As a result, a large number of fertiliser factories had been either already commissioned or in the process of commissioning. The nitrogen fertiliser wastewater emerging out of the industries pose a serious problem of water pollution in the already polluted rivers, lakes and shore waters. The ecological balance of waters get disturbed. Consequently the water bodies become unsuitable for any useful purpose. Activities of vital economic importance such as navigation, irrigation and fishing are adversely affected

So the Water Pollution Control Boards of Central Government and State Governments have put stringent quality standards for the effluents of industries.

1.4. Research on Fertiliser Effluent Treatment:

Majority of fertiliser industries in India are oriented towards producing nitrogenous fertilisers. The methods of effluent treatment that are accepted by more advanced nations cannot be adopted as such for Indian conditions for reasons of economics. Air-stripping, nitrification-denitrification, ion exchange, electrochemical precipitation, membrane processes are but few of the methods accepted as feasible by various western affluent nations. For one reason or other such technologies are not suitable for our country. Moreover India is a country where solar energy is abundantly available almost throughout the year. Availability of land is also not scarce. It is in this perspective that a new low cost technology should be developed for treating the nitrogenous wastes of fertiliser industry.

Recent developments in wastewater technology using algae and bacteria have opened a new era of promise. It has been demonstrated that domestic wastewater can be treated effectively using this method (McGriff, 1970 ; John, 1976). The feasibility of algal bacterial system for treating domestic wastewater under natural sunlight had been successfully accomplished by John (1976) at I.I.T. Kanpur, India. It is this process that was

tried in the treatment of nitrogenous fertiliser effluents. The ammonia concentration being high, larger biomass concentration, feeding of organic carbon for bacterial growth, aeration to keep the biomass in suspension and for successful growth and finally pH adjustment for efficient bioextraction of ammonia were few of the innovations successfully attempted in this research programme.

Theoretical background for the growth of algae and bacteria have been kept in mind while deriving and proposing the present system. The proposed system has been found effective in the removal of organics and nutrients and the resulting sludge was settling well. The settled algal bacterial biomass could be used for digestion or composting, thereby recovering the trapped nitrogen in them. Incidentally a new mechanism has been revealed during the research. When ammonia N concentration is large, pH in the range of 8.0-8.3 and if aeration is sufficient, there seems to be a "Luxury uptake of nitrogen" by the flocculating algal bacterial biomass. This was evidenced by sludge analysis and nitrogen mass balance. This new finding might go a long way in further improving the existing processes where algal bacterial cultures are used.

2. ORIGIN AND TREATMENT METHODS OF HIGH NITROGENOUS WASTES

2.1. General:

In a developing country like India with immense potentialities in the field of agricultural production, it is only in the fitness of things that the Government of India have been giving paramount importance for fertiliser manufacture. Even though tremendous strides have been made in the field of fertiliser manufacture, it is still a herculean task for the country to cope up with ever increasing demands. The exigency to produce more in the agricultural sector stems from the grim reality of exploding population and availability of limited area of cultivable land. Modern agricultural research has opened new vistas for maximising the output by using hybrid varities of seeds, intensive use of fertilisers and abundant irrigation facilities. So it stands to reason how much vital it is to

establish more and more fertiliser factories to build up a welfare state self-sufficient in food and clothing. Realising the immense responsibilities vested on their shoulders, the Government of India have taken up the challenge with all seriousness.

2.2. Historical Background:

A perusal of the history of fertiliser manufacture in India gives a hopeful picture. With a meagre quantity of fertiliser production of 24 thousand tons per annum before 1951, it has touched an all time high figure of 3.9 million tonnes in 1977. Chari (1976) has given a detailed account of the status of fertiliser production in India. Table 1 would illustrate the facts.

Table 1 - Fertiliser Units and Their Annual Capacity

Year	Name of Unit	Capacity, Tonnes	
		N	P_2O_5
Before 1951	FACT, Alwaye	20,300	3,300
1951-61	FCI, Sindri	90,000	-
	NCJM, Varanasi	10,000	-
	FCI, Nangal	80,000	-
1961-71	FACT-expansion, Alwaye	46,700	22,400
	FCI, Trombay	81,000	36,000
	FCI, Gorakhpur	80,000	-

Continued..

Table 1 (continued)

Year	Name of Unit	Capacity, Tonnes	
		N	P_2O_5
	FCI, Namrup	45,000	-
	NLC, Neyveli	70,000	-
	HSL, Rourkela	120,000	-
	EID Parry, Ennore	16,000	10,300
	GSFC, Baroda	216,000	50,000
	CFL, Vizag	80,000	73,000
	IEL, Kanpur	200,000	-
	SCI, Kota	110,000	-
1971-77	FACT-expansion, Alway	15,000	10,500
	SCI-expansion, Kota	42,000	-
	CFL-expansion, Vizag	35,000	-
	FCI-expansion, Gorakhpur	51,000	-
	MFL, Madras	176,000	112,000
	ZACL, Goa	170,000	42,000
	FACT, Cochin	152,000	-
	FCI, Durgapur	152,000	-
	IFFCO, Kalol and Kandla	215,000	18,000
	FCI, Trombay	32,000	18,000
	SPIC, Tutucorin	258,000	51,000
	MCFL, Mangalore	160,000	-
	FCI, Barauni	152,000	-
	KCL, Khetri	-	90,000
	FCI-expansion, Namrup	152,000	-
	Byproduct Ammonium Sulphate	30,000	-
	SSP Units	-	227,000
	TSP and Pelfo	-	20,000
1977-78	FACT-Phase II, Cochin	40,000	114,000
	FCI, Sindri	-	156,000
	FCI, Talcher	228,000	-
	FCI-expansion, Nangal	152,000	-
	FCI, Ramagundam	228,000	-
	FCI, Trombay	75,000	75,000
1978-79	FCI-modernisation, Sindri	129,000	-
	FCI, Haldia	152,000	75,000
	Maharashtra Cooperative	51,000	-
	NFL, Bhatinda	235,000	-
	NFL, Panipat	235,000	-
	HZL, Udaipur	-	26,000

Continued ...

Table 1 (continued)

Year	Name of Unit	Capacity, Tonnes	
		N	P_2O_5
1979-80	IFFCO, Phulpur	228,000	-
	FCI, Trombay	130,000	-
1980-81	GSFC, Baroda	243,000	-
1981-82	Narguna	228,000	-
1982-83	Narguna	-	82,000
1983-84	Total	5,379,000	1,451,000

Fertilisers produced in India can be broadly classified as nitrogenous and phosphatic, the former being produced in enormous quantities than the latter. Important among nitrogenous fertilisers include urea, ammonium sulphate, ammonium nitrate, ammonium chloride and ammonium phosphate. Complex fertilisers containing both nitrogen and phosphorus are also produced in certain factories. Modern technology has brought in many innovations and process modifications in the manufacture of fertilisers. Most of the earlier units were designed to manufacture ammonium sulphate and ammonium nitrate. But the recent trend is mainly in the manufacture of urea.

Changes in the raw materials and modern developments in the manufacturing processes have brought in their wake the problem of treatment and disposal of a number of new and highly toxic effluents. The question of disposal of ammonia and other ammonium

salts in the effluents exists still as before. The task of pollution control in complex fertiliser units has become more intricate. Hitherto the effluents from the nitrogenous fertiliser factories discharging into streams were considered primarily responsible for fish kills, eutrophication and degradation of water quality. But the addition of phosphate bearing wastes to the nitrogenous effluents has accentuated the problem of eutrophication to such an extent that receiving streams can no longer be put to any useful purposes. NEERI, India had carried out waste surveys in a number of fertiliser factories in different parts of the country and has suggested measures for controlling and disposal of the effluents (Chakravarty et.al., 1971).

2.3. Manufacturing Processes of Nitrogenous Fertilisers:

Ammonia, which is the principal intermediate in the manufacture of all nitrogenous fertilisers is obtained as a product of Haeber-Bosch process or as a by-product from coke ovens. In producing ammonium sulphate, ammonia is reacted with sulphuric acid. However in the manufacture of urea, carbon dioxide also is required. The basic processes of manufacture of urea are the following:

(a) Gassification of naphtha or natural gas in the presence of steam and air to obtain carbon dioxide, hydrogen and nitrogen.

(b) Separation and purification of carbon dioxide.

(c) Synthesis of ammonia from hydrogen and nitrogen.

(d) Synthesis of urea from ammonia and carbon dioxide.

2.3.1. Gassification:

There are two stages in the gassification of naphtha or natural gas. The first stage of gassification can be achieved by two methods. In the partial oxidation process the hydrocarbon is completely oxidised with air or oxygen, producing a high percentage of carbon monoxide and low percentage of hydrogen. A substantial quantity of uncombusted carbon is released as soot. In the steam reforming process, steam and air are used for gassification of the resulting hydrocarbon. In this method the gas contains hydrogen, carbon monoxide, carbon dioxide and nitrogen. In the second stage of gassification known as shift conversion process the carbon monoxide is converted to carbon dioxide besides enriching the gas mixture with additional quantities of hydrogen. Figure 1 illustrates the process of gassification (Chakravarty et.al., 1971).

2.3.2. Carbon Dioxide Separation and Purification:

The carbon dioxide separators are intended to separate the carbon dioxide from the gas mixture coming out of shift convertors. The separation towers contain solutions of various organic and inorganic compounds such as monoethanolamine, diethanolamine, potassium carbonate, arsenic and caustic soda. One or more of

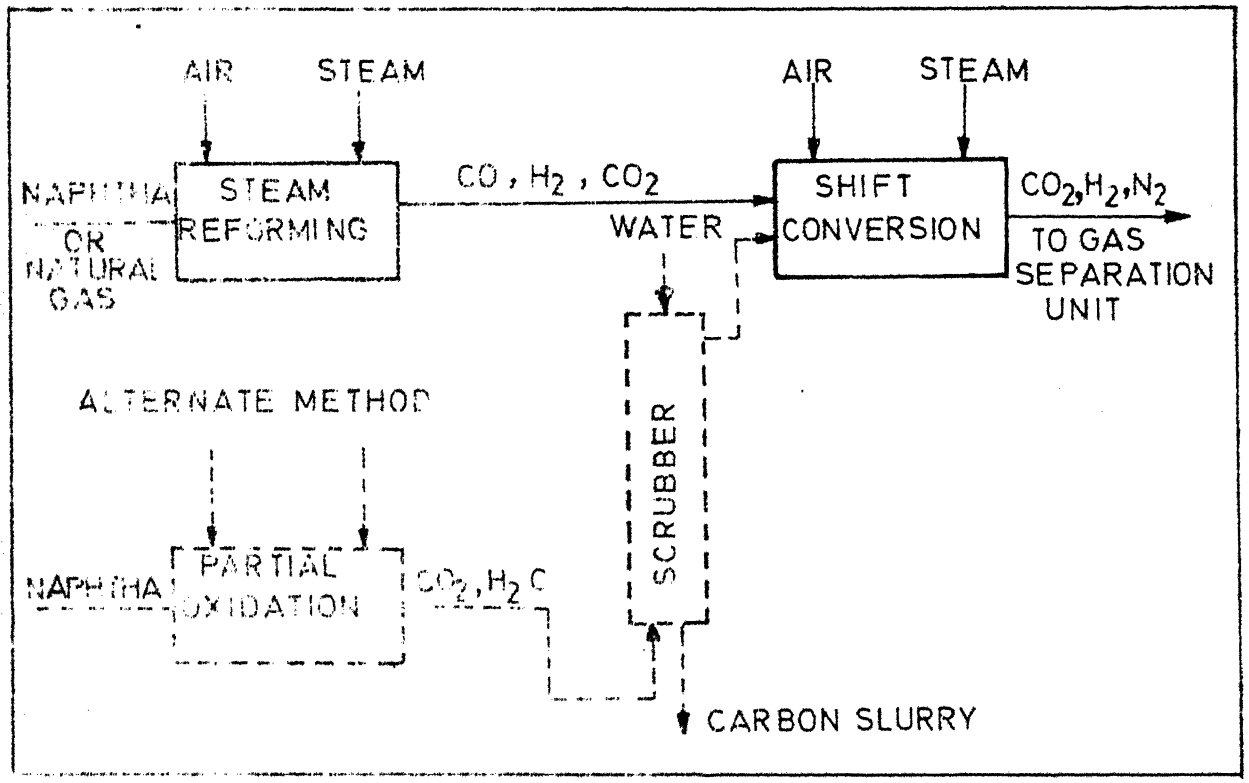


FIG.1 GASSIFICATION PLANT

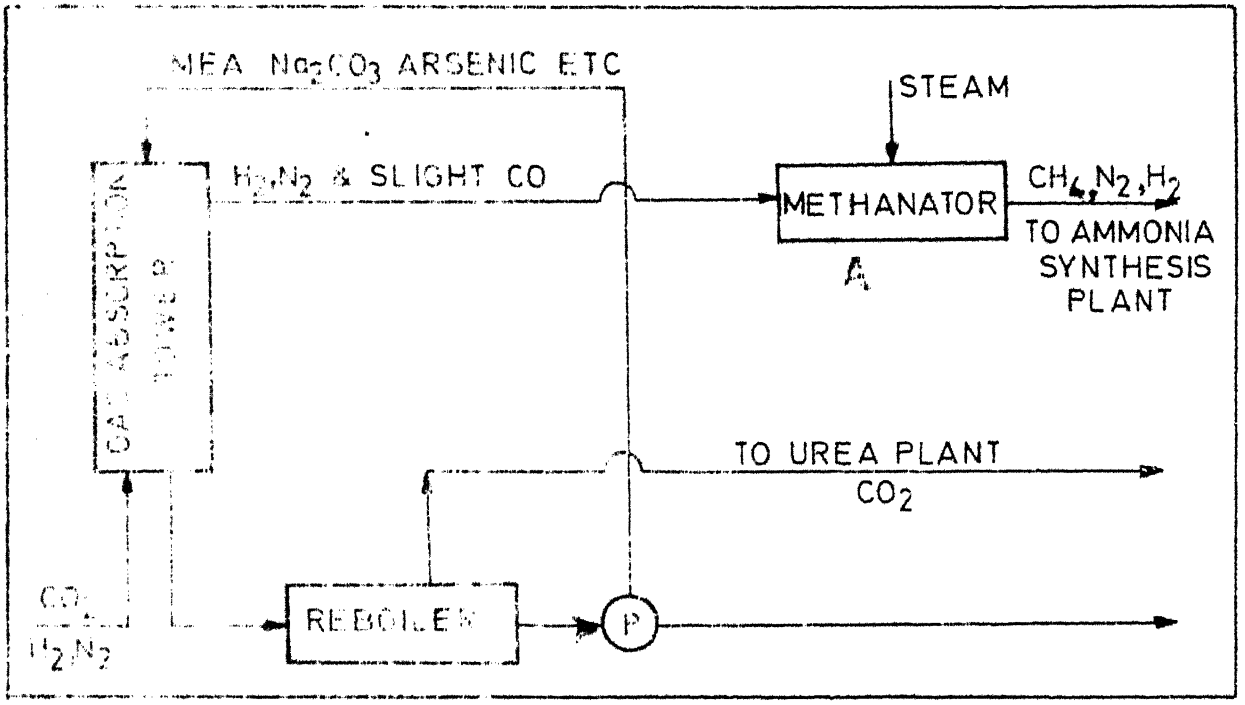


FIG.2 GAS SEPARATION PLANT

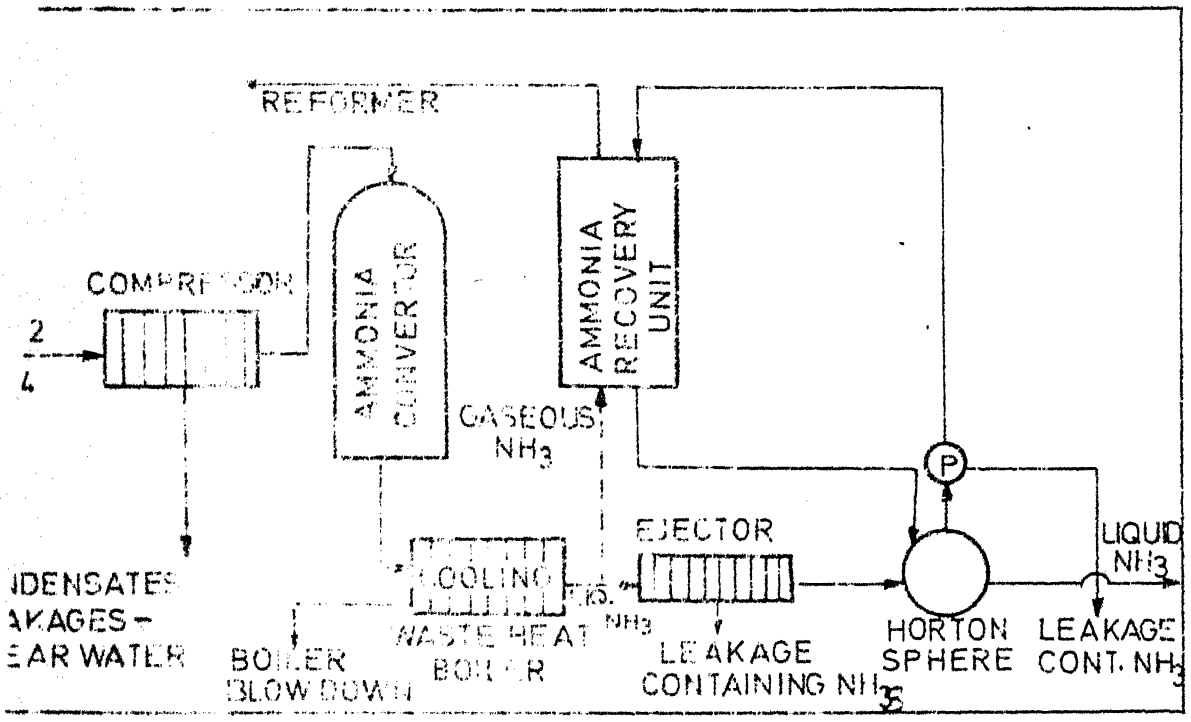


FIG.3 AMMONIA SYNTHESIS UNIT

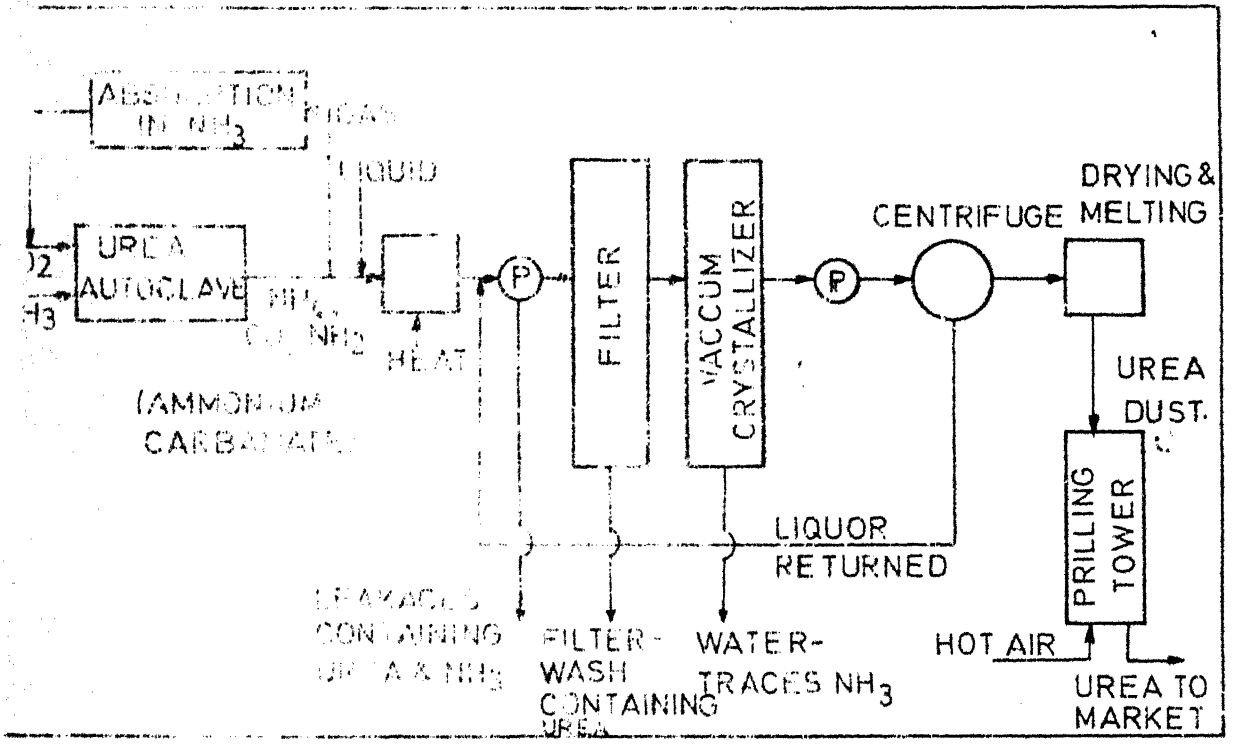


FIG.4 UREA SYNTHESIS PLANT

the above-mentioned chemicals are used depending on the system. The layout of gas separation plant is shown in Figure 2.

2.3.3. Carbon Monoxide Removal:

The gas stream from carbon dioxide separators are composed of hydrogen and nitrogen. A small amount of carbon monoxide, which may be present, is eliminated in methanator and is purged out of the ammonia absorber after ammonia synthesis.

2.3.4. Ammonia Synthesis:

The gases from the methanator are compressed in high pressure compressors and recirculated through convertors filled with catalysts. This results in the formation of ammonia. On cooling ammonia liquifies and is separated from the gases and stored in what are called Horton spheres. From Horton spheres the liquid ammonia is drawn for further processes. The gases go further to an ammonia recovery plant. The purge gas sent out to the reformer is used as fuel as it contains methane. The layout is shown in Figure 3.

2.3.5. Urea Synthesis:

In a plant known as urea autoclave carbon dioxide and ammonia are brought to reaction at high pressure, when ammonium carbamate is formed. This ammonium carbamate is further decomposed in stages to form urea which remains in solution. The urea solution is filtered in diatomaceous earth or other filters and

crystallised in vacuum crystallisers. These crystals are separated from the mother liquor, which is again recycled back. The gases which have not reacted are also absorbed in ammonia and recycled back to the urea autoclave. The urea crystals are dried, remelted and conveyed to the top of prilling tower. Here the molten mass is allowed to fall against a rising current of hot air to produce spherical prills of urea, each 2-3 mm in diameter. The prills are collected and stored in silos for packaging and marketing. The process layout is illustrated in Figure 4.

2.3.6. Manufacture of Ammonium Sulphate:

Ammonium sulphate is manufactured either by reacting anhydrous ammonia with sulphuric acid or by the double decomposition of ammonium carbonate and calcium sulphate. In some plants gypsum is obtained from mines whereas in others the calcium sulphate sludge is obtained as a by-product of phosphoric acid manufacture.

2.3.7. Manufacture of Calcium Ammonium Nitrate:

In this process a part of ammonia previously prepared is oxidised with oxygen-enriched air and is absorbed in water to form nitric acid. This nitric acid is reacted with another part of ammonia to form ammonium nitrate. Then the solution of ammonium nitrate is concentrated in evaporators, mixed with powdered limestone and dried to form granules of calcium ammonium nitrate.

2.4. Sources and Nature of Effluents:

The following are the sources and nature of effluents from ammonia and urea plants.

2.4.1. Ammonia Plant Effluent:

Ammonia plant effluents are composed of process condensates, aqua ammonia, leakages and flow from carbon dioxide removal system.

2.4.1.1. Process Condensate: A considerable portion of steam used in the reformers of the ammonia plant has to be removed after it heats up the absorbant solution in the carbon dioxide removal system. This condensate contains approximately 400-600 ppm of ammonia N besides carbon dioxide, methanol and other organic compounds (Pillai, 1976). This condensate is unfit for reuse in the process in the normal circumstances. Most of the waste is likely to contain ammonia N.

2.4.1.2. Aqua Ammonia: A certain quantity of dilute ammonia produced during initial reduction of ammonia converter catalysts has to be drained off. This mostly consists of free ammonia N. In addition leakages from high pressure compressors and circulating pumps also contain ammonia N.

2.4.1.3. Effluents from Carbon Dioxide Removal System: The impurities in the effluent will depend upon the type of absorbent solution used such as GV, Bonfield, Glycene, Carsol, MEA etc. In these solutions arsenic, vanadium, chromium etc. are used as

promoters or inhibitors. These inorganic and heavy metal compounds are highly toxic. They are normally handled separately and segregated from other effluents.

2.4.2. Urea Effluents:

The sources of effluents from urea plants are leakages from high and low pressure pumps, blow down from cooling water system when this water is used for vacuum generation in the crystalliser, accidental spillages and washings. The main constituents of the effluent are ammonia N and urea N.

2.4.3. Utility Plant Effluents:

Oil contamination comes from spillages from lube oil, furnace oil and naphtha of which the latter is inflammable too. Such materials should be removed prior to discharge by liquid-liquid separation. Cooling towers have to be blown down continuously or intermittently. This effluent will contain the inhibitors in cooling water. Sodium chromate is one of the main contaminants.

Certain urea plants use cooling water system which is contaminated with ammonia and urea. Blow down from such cooling towers, in addition to ammonia and urea contamination, will also contain nitrites and nitrates. Effluent water from water treatment plant may contain dilute solution of acids and alkalies used for the regeneration of demineralising resins.

2.5. Volumes and Characteristics of Wastes:

It is apparent from the foregoing paragraphs that a number of substances in the form of processing chemicals, intermediates and final products used would be discharged with the effluents in varying quantities, depending on the process and stringent standards observed in the housekeeping. The volumes of flow of different effluents in a fertiliser factory greatly vary, depending on the process involved. While some wastewaters are discharged intermittently, a number of others have continuous flows and they represent effluents containing toxic materials. Another significant point to be observed is that two plants producing the same quantity of urea or other products per day and having the same basic processes of manufacture discharge widely different volumes of process wastes. Hence it is very difficult to establish an average value for any of the waste fractions and its characteristics. Nevertheless it could be possible to arrive at a reasonable range both for volumes and the characteristics. Chakravarty *et al.* (1971) had documented about the volume and characteristics of wastes from fertiliser industries based on the data collected by CPHERI as presented in Table 2.

Mohanrao *et al.* (1973) reported that distribution of ammonia N and urea N for different factories is different. Table 3 illustrates these facts. Hence only certain average values as in Table 3 could be utilised for the purposes of laboratory studies.

Table 2 - Volumes of Flow of Effluents and Load of Important Waste Constituents in a Fertiliser Plant (Chakravarty et al., 1971)

Nitrogenous Fertiliser Plant

Type of Waste Constituents	Loss in kg/ tonnes NH_3/ day	Effluent gpd/tonnes NH_3/ day
1. Carbon slurry		
(i) Total carbon	1.45-1.47	430-500
2. Scrubber wastes		
(i) K_2CO_3	0.065-0.074	5-20
(ii) NaOH	0.02-0.03	10-30
(iii) As_2O_3	0.052-0.055	20-160
(iv) MEA	0.038-0.28	10-160
3. Process wastes in ammonia and ammonium salt plants		
(i) Ammonia as NH_3	4.65-10	456-600
4. Process wastes in urea plant		
(i) Urea as $CO(NH_2)_2$	15-17.5	1100-1500

Table 3 - Distribution of Ammonia N and Urea N

Factory	Percentages of Distribution	
	Ammonia N	Urea N
A	79	21
B	67	33

2.6. Impact of Pollution on Water Bodies:

Almost all the components present in fertiliser factory effluents can affect the receiving water in one way or other. Acidic and alkaline wastes can substantially destroy the normal aquatic organisms of a stream or lake and inhibit its normal self purification property. Moreover these constituents are toxic to fish and other aquatic organisms. Normally fertiliser factory effluents are alkaline in nature. This is primarily attributable to the presence of ammonia, potassium carbonate and caustic soda. Ammonia even in very small concentration is toxic to fish. Southgate (1952) observed that 1.2-3.0 mg/l of ammonia could be lethal to fish. Doudoroff et al. (1950) had demonstrated that the toxicity of ammonia is dependent primarily on the undissociated NH_4OH and nonionic ammonia. Loehr (1974) indicated that ammonia toxicity increases markedly when the quantities of dissolved oxygen are reduced. Field studies (1968) by Federal Water Pollution Control Administration of United States led to

the conclusion that at pH levels of 8.0 and above the total ammonia N concentration should not exceed 1.5 mg/l. It had been reported that a concentration of 2.5 mg/l of total ammonia N is actually toxic both for fresh water and marine fish. ISI (1963) had prescribed a limit of 1.2 mg/l of ammonia N in a river water used for pisciculture.

Above all these, nitrogen compounds are nutrients and may cause undesirable algal growths. The amines released along the wastewater are not only toxic to aquatic life but exert a high oxygen and chlorine demand. Hence it follows that a river water having sufficiently high ammonia and amine concentration would require longer contact period and larger doses of chlorine for satisfactory disinfection in a water treatment plant. Gillette et al. (1952) observed that MEA in excess of 10 mg/l is fatal to fish. Pillai (1976) had indicated that in normal circumstances urea is not toxic to fish up to a concentration of 16000-30000 mg/l. However, under anaerobic conditions or at high pH urea decomposes to ammonia N and carbon dioxide and becomes toxic.

Arsenic is extremely toxic to fish and other aquatic organisms. Shen et al. (1965) had reported that the presence of 1.0 mg/l of arsenic in drinking water in Taiwan had caused blackfoot diseases among the consumers. Arsenic is a cumulative poison and consistent intake have caused fatalities as had been documented by Wyllie (1939). ISI (1963) has stipulated a limit of 1.0 mg/l of arsenic in industrial effluents discharged into inland surface waters.

Flourides which are found in the effluents of phosphatic fertilisers, are also considered to be injurious to health above certain concentrations. ISI (1963) has prescribed a limit of 2.0 mg/l of flourides as F in effluents discharged into water courses. Chakravarty et al. (1971) quoted Makee et al. having observed that hatching of fish eggs were adversely affected by flouride laden water. However no mention is made of any immediate injury to fish.

Phosphates do not seem to have direct toxic effects on aquatic organisms. On the contrary they play a vital role in the eutrophication of receiving waters. The quantity of phosphorus necessary for algal blooms is so small that it would be rather difficult to control the problem. Sawyer (1952) had documented that the minimum requirements of phosphorus as $PO_4^{\equiv}$ and nitrogen as N for production of algal bloom in a body of water are 0.015 mg/l and 0.067 mg/l respectively. It would be worthwhile if a perusal of the Indian Standards as given in Table 4 is done before ending this paragraph.

2.7. Segregation and Treatment of Wastes:

The major contaminants present in fertiliser wastes which need some treatment or other include oil, arsenic, phosphates, flourides, ammonia and urea. Oil and grease derived from compressor section are generally in excess of permissible limits and interfere with subsequent treatment processes including biological treatments.

Table 4 - Relevant Tolerance Limits for Effluents Discharged into Inland Surface Waters (ISI, 1963)

Characteristics	Tolerance Limit
1. In IS: 2490-1963	
(i) Total suspended solids mg/l maximum	100
(ii) pH	5.5-9.0
(iii) Temperature	Shall not exceed 40°C in any section of the stream within 15 m downstream of effluent outlet
(iv) Cyanides as CN mg/l maximum	0.2
(v) Flourides as F mg/l maximum	2.0
(vi) Oils, grease mg/l maximum	10.0
(vii) Phenolic compounds mg/l maximum	1.0
(viii) Arsenic as As mg/l maximum	1.0
2. Tolerance limit/modified in the revision of IS: 2490	
(i) Arsenic as As mg/l maximum	0.2
(ii) Ammonia as N mg/l maximum	50.0

An oil separator of properly designed capacity is an inevitable entity for the segregation of this impurity from other wastes. Arsenic wastes are segregated and concentrated by evaporation to obtain solid sludge. This sludge is disposed off safely. The acidic wastes from demineraliser units are stored in acid resistant tanks and treated separately and disposed off.

Phosphate and flouride wastes emanating from phosphatic fertiliser manufacture units are also segregated, treated and disposed. Lime precipitation for phosphates and flourides is an effective method. Arceivala et al. (1972) had made it clear that for further treatment of phosphates and flourides they may be mixed with other wastes and treated.

Ammonia, ammonium salts and urea are among the most important contaminants of a fertiliser factory effluent. Bhalerao et al. (1973) studied various methods of segregation and treatment of nitrogenous fertiliser factory effluents. A typical diagrammatic sketch detailing the segregation of wastes is shown in Figure 5. From this layout it is clear that all toxic and other wastes such as arsenic, acids, oils and grease are segregated from the source itself and treated separately. Thus nitrogenous wastes alone are brought under one treatment plant; where anyone of the various methods could be applied.

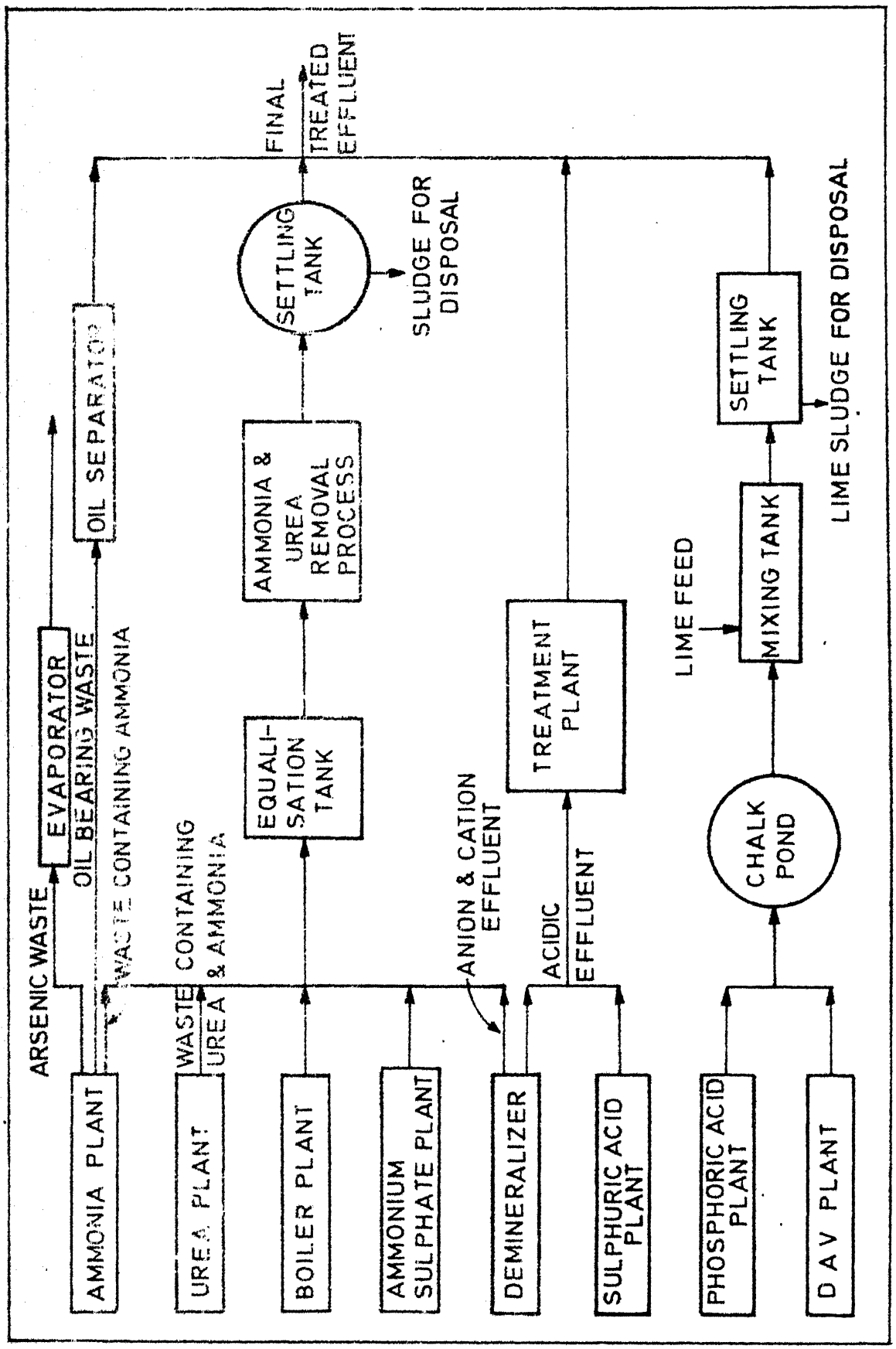


FIG. 5 LAY OUT SHOWING SEGREGATION AND TREATMENT

.8. Ammonia and Urea Removal Techniques:

arious techniques have been proposed and tried for the removal of ammonia and urea. However only very few are found to be successful in the field. This might be either due to prohibitive costs of installation and operation or due to inefficient working or other inherent defects. The following are the ammonia removal methods presently available:

(a) Air stripping
(b) Steam stripping
(c) Ion exchange process
(d) Break point chlorination
(e) Electrodialysis
(f) Reverse osmosis
(g) Electrochemical precipitation
(h) Simple lagooning
(i) Biochemical nitrification-denitrification
(j) Algal pond treatments
(k) Enzyme process.

It would be only appropriate if the above-mentioned methods are discussed in brief before going into the details of the new process developed.

2.8.1. Air Stripping:

2.8.1.1. Theoretical Background: From Henry's law of partial pressures and Dalton's law it can be seen that under equilibrium conditions the amount of a gas dissolved in water from a mixture

of gases is proportional to its partial pressure. Partial pressure is expressed as:

$$C = H.P. \qquad 2.1$$

where, C = Concentration of soluble gas in liquid in unionised form (mg/l)

P = Partial pressure of the soluble gas in gas phase (atm)

H = Solubility coefficient which depends on temperature (mg/l atm).

Gases such as carbon dioxide and ammonia deviate from Henry's law at ordinary temperatures due to their reaction with water. However at higher temperatures the deviation is less. In air-stripping air bubbles provide sufficient area for diffusion of ammonia into the bubble and this carries away the ammonia with it. Ammonia removal by air-stripping is a modification of the aeration process used for the removal of gases from water. The same principles utilised in the design of cooling towers are applicable to air-stripping of ammonia as well.

The important factors controlling the design and operation of stripping process are the pH of the waste effluent, the rate of mass transfer and the ratio of air to liquid requirements. Ammonium ions in wastewater exist in equilibrium with ammonia and hydrogen ions thus:

$$NH_4^+ \rightleftharpoons NH_3 + H^+ \qquad 2.2$$

When pH is increased above 7.0 the equilibrium is shifted to the right. At pH 10.0, over 85 percent of ammonia may be liberated as gas by agitating the wastewater in the presence of air. O'Farrel (1972) had shown that when pH is between 10.0-11.0 the excess hydroxyl ions converts the ammonium ions to ammonium hydroxide thus:

$$NH_4^+ + OH^- \rightleftharpoons NH_4OH \rightleftharpoons NH_3 + H_2O \qquad 2.3$$

Ammonia is over 1000 times more soluble in water than carbon dioxide and the volatility decreases considerably with decrease in temperature. These factors pose serious problems in air-stripping. To obviate these hurdles in effective air-stripping, warm temperature and large air to liquid ratios become imperative. Bhattacharrya et al. (1973) in a review have given Bayley's equation for the calculation of volume of air required to bring about any specified reduction in concentration of ammonia in alkaline media in a continuous stripper. The equation is:

$$\frac{C_1 + C_2}{C_1} = \frac{10^3 \rho \cdot Q \cdot t_R}{V \cdot H} \qquad 2.4$$

where, C_1, C_2 = Concentration of ammonia in the influent and effluent respectively (mg/l)

ρ = Density of ammonia vapour (g/l atm)

Q = Rate of flow of air, (l/hr)

t_R = Residence time in stripping unit (hr)

V = Volume of liquid in stripping tower

H = Reciprocal of Henry's constant.

2.8.1.2. Works Done on Air-Stripping: Ample works had been documented on air-stripping for ammonia removal from sewage effluents. However comparatively less amount of work has been done on air-stripping of high nitrogenous wastes. Reeves et al. (1972) had reported that the ammonia N in domestic wastes is in the range of 10-50 mg/l. Lakshminarayana et al. (1972) observed that the free ammonia N level of raw sewage ranges from 27-59 ppm. O'Farrel et al. (1972) found that air-stripping in cross flow cooling towers at water temperature of 26.6°C removed 90 percent of ammonia N from a non-nitrified lime clarified secondary effluent at pH 11.5 with 3750 l/l of air flow. Heavy calcium carbonate scales were formed within the tower from carbon dioxide in the air and excess calcium in the wastewater. However at pH 9.7 the efficiency of removal was reduced by 34 percent. Again when temperature was brought down to 5.5°C the removal efficiency dropped by 30 percent. Reeves et al. (1972) reported that Culp inferred that the cost of air-stripping is $ 0.015/gal for 95 percent removal. This, however, is exclusive of other cleaning and other maintenance costs. Because of the high pH, scale formed readily on the closely packed media in the towers. This scale was removed frequently by acid washing and mechanical scrubbing.

Reeves et al. (1972) quoted Kuhn to have found out that the air to water ratio for the best performance at pH 11.0 to be 205 cum/min/l/day. Rao et al. (1971) observed that air-stripping is

effective for the removal of ammonia from fertiliser wastes. Jain et al. (1977) reported that at a pH of 12.1 and air flow of 13.7 l/min ammonia N and urea N removal is almost 100 percent in 36 hours. From a comprehensive study on the removal of ammonia N from fertiliser wastes, Bhalerao et al. (1973) arrived at the following facts illustrated in Table 5. The flow of effluent recorded was 3 mgd.

Table 5 - Comparative Cost of Air-Stripping

Method	Capital Cost Rs. in Lakhs	Annual Running Cost Rs. in Lakhs
Using lime for pH increase	44.00	23.00
Using caustic for pH increase	33.00	43.00

Further they concluded that air-stripping works out to be the costliest of the methods tried.

Roesler et al. (1971) presented a computer model for the design of ammonia N stripping and cooling towers. According to them absorption of carbon dioxide from the air and subsequent precipitation of carbonates and the biological nitrification of ammonia in the aerobic towers are the two major problems encountered. Bhattacharrya et al. (1972) quoted McKinney having emphasised about the disadvantages of air-stripping. Besides biological

oxidation of ammonia and scale formation, air-stripping, is said to be quite impractical. He further reported that the ammonia that is released in the air and got absorbed would quickly be returned to the water environment with no real gain.

Pillai (1976) had gone on record to say that there are but few stripping towers in fertiliser plants in India. In his opinion, the wood used in the towers get deteriorated due to high pH of the solution and also due to nitrites and nitrates formed during air-stripping. The pH of the stripped effluent being much above the specified limits, pH adjustment would involve in an additional process thereby increasing the cost still further.

2.8.2. High Pressure Steam Stripping:

In the process of steam stripping, steam is directly injected to raise the temperature and pressure. Moreover demineralised water has to be used for the stripper feed solution. Pillai (1976) reported that several ammonia strippers using steam are operating for the treatment of sour waters in petroleum refineries. API reports indicate that many steam strippers reduce the ammonia N and urea N levels to less than 100 mg/l. Thermal hydrolysis of urea was tried by Smit et al. (1976). They found that the hydrolysis proceeds according to the gross equation:

$$CO(NH_2)_2 + H_2O \rightarrow 2NH_3 + CO_2 \qquad 2.5$$

High temperature steam or air was injected into the reactor. The effluent they obtained after thermal hydrolysis contained 200 mg/l urea N and 50 mg/l ammonia N. The process is reported to be costly. Pillai (1976) reported that a steam stripper for a feed of 50 l/hr would cost about 110 lakhs.

2.8.3. Ion Exchange Process:

Many have tried ion exchange process for ammonia N removal. Reviewing Baumann's work, Reeves et al. (1972) reported that the process involves huge expenditure and is very complex in nature. The biggest factor to be resolved in the ion exchange process is the disposal of concentrated brine solution with high ammonia N content. Ames, as quoted by Reeves et al. (1972), could regenerate clinoptilolite by relatively inexpensive lime solution. He observed that clinoptilolite has preference for NH_4^+ to Ca^{++} ions. Mercer (1970) obtained 99 percent removal with selective ion exchange process while treating clarified secondary effluent containing 10-19 mg/l ammonia N. He reported that 200-300 column volumes of the effluent could be treated before regeneration.

From their study on tertiary treatment of weak ammonia liquor, Cousins et al. (1972) concluded that hydrogen ion exchange is impractical because of resin fouling. Weak ammonia liquor is the waste product obtained during the distructive distillation of coal. The waste contains 4000-5000 mg/l ammonia N besides other impurities. Sulphonated styrene divinyl benzene copolymer resin was found to produce an effluent with 40-50 mg/l ammonia N from

an influent concentration of 1250 mg/l. The authors observed that the process is impracticable. According to Cohen, as quoted by Reeves et al. (1972), the cost of ion exchange process for treating domestic wastes works out to be $ 0.17-0.18/1000 gal.

2.8.4. Break Point Chlorination:

The principle involved in this process is chlorinating nitrogenous effluents to oxidise ammonia and also to reduce nitrites and nitrates into gaseous nitrogen products. With molar ratio of chlorine : ammonia up to 1 : 1 monochloramines and dichloramines are formed. With further addition of chlorine the chloramines gradually decompose and at a molar ratio of chlorine : ammonia of 2 : 1, break point occurs showing negligible chloramine residuals. After break point HOCl remains in the solution. The optimum oxidation occurs at pH 7.0-8.0. The reactions are the following:

$$2NH_3 + 3Cl_2 \rightarrow N_2 + 6HCl \qquad 2.6$$

$$4NH_2Cl + 3Cl_2 + H_2O \rightarrow N_2 + N_2O + 10HCl \qquad 2.7$$

$$2NHCl_2 + H_2O \rightarrow N_2O + 4HCl \qquad 2.8$$

$$3HOCl + 2NHCl_2 + H_2O \rightarrow 2NO_2 + 7HCl \qquad 2.9$$

It is presumed that nitrogen, nitrous oxide and nitrogen dioxide are the possible gaseous products. O'Farrel, as quoted by Bhattacharrya et al. (1973), found that up to 10 mg of chlorine is required for reacting with 1.0 mg of ammonia N. High

chlorine demand is the biggest limiting factor for the use of this process for the bulk removal of ammonia N from industrial wastes.

2.8.5. Electrodialysis:

Electric current induces partial separation of components in an ionic solution. The separation is accomplished by alternatively placing cation and anion selective membranes across the current path. When current is applied, the electrically attracted cations pass through cation exchange membrane in one direction and the anions pass through the anion exchange membrane in the other direction. This being a very delicate process is unsuitable for high nitrogenous wastewaters. Weber (1972) quoted Korngold *et al.* having reported that chemical precipitation of salts like calcium carbonate on the membranes and clogging of the membranes by colloidal impurities render the process more vulnerable in the treatment of wastes. So pretreatment by very sophisticated methods becomes imperative.

2.8.6. Reverse Osmosis:

Osmosis is defined as the spontaneous transport of a solvent from a dilute solution to a concentrated solution across an ideal semi-permeable membrane which impedes passage of solute but allows the solvent to flow. Reverse of this, called reverse osmosis could be accomplished by exerting a pressure above osmotic pressure on a concentrated solution side of the membrane. Then

pure solvent will pass from solution into the solvent. The mechanisms involved in the reverse osmosis include seiving action, surface tension and hydrogen bonding by cellulose acetate membranes.

It is reported by Weber (1973) that encouraging results were obtained in the pilot plant studies on acid mine and sanitary wastes. The membrane being made of an organic ester, the optimum pH range of operation should lie between 3.0-7.0. Bhattacharrya et al. (1973) reported that at pH values less than 7.0 ammonia N present as ammonium ion is retarded by the thick membranes. However the penetration of ammonia N (NH_3) could not be prevented. Problems associated with reverse osmosis include concentration polarisation and membrane fouling.

2.8.7. Electrochemical Precipitation:

Clesceri (1968) reported that Foyan had developed an electrochemical method in Norway for precipitation of ammonia N from raw wastewater. In this method wastewater is mixed with sea water and is passed into a single cell containing carbon electrodes. Due to relative densities of the sea water and the mixture, the former accumulates in the anode area at the bottom of the cell and the latter at the cathode area near the top of the cell. The current raises the pH at the cathode thereby precipitating ammonia as $MgNH_4PO_4$ along with magnesium hydroxide. Hydrogen bubbles generated at the cathode lift the sludge to the surface

when it is skimmed off. Chlorine developed at the anode disinfects the effluent. The resultant solution is rejected. However its applicability to the high nitrogenous wastes had not been reported to the best knowledge of the author.

2.8.8. Simple Lagooning:

Pilot plant studies by NEERI,(India)for treating nitrogenous wastes by simple lagooning has been documented by Bhalerao et al. (1973). Lime was used to raise the pH to 11.0. Ammonia reduction in a period of 24 hours ranged from 44 percent to 61 percent. However after 48 hours ammonia N was found to increase due to the gradual transformation of urea into ammonia. Hence they inferred that urea plant wastes aught to be retained in separate lagoons for a sufficiently long period prior to discharg into simple lagoons with pH adjustment. No further details are available.

2.8.9. Biochemical Nitrification-Denitrification:

The principles involved in this method are to convert the ammonia N into nitrites and nitrates by highly specific groups of aerobic bacteria and then converting the nitrites and nitrates by another group of anaerobic bacteria into gaseous nitrogen products. This process consists of two steps: nitrification and denitrification.

2.8.9.1. Biochemical Nitrification: Biochemical conversion of ammonia to nitrites and nitrates by highly specific groups of aerobic, autotrophic bacteria Nitrosomonas and Nitrobacter is

called nitrification. Haug et al. (1972) postulated the overall reaction of nitrification and assimilation approximately as follows:

Nitrosomonas:

$$55NH_4^+ + 5CO_2 + 76O_2 \rightarrow C_5H_7O_2N + 54NO_2^- + 52H_2O + 109H^+ \qquad 2.10$$

Nitrobacter:

$$400NO_2^- + 5CO_2 + NH_4^+ + 195O_2 + 2H_2O \rightarrow C_5H_7O_2N + 400NO_3^- + H^+ \qquad 2.11$$

On the basis of the above nitrification reactions 20 mg/l ammonia N would produce about 3 mg/l Nitrosomonas and about 0.5 mg/l Nitrobacter while consuming 85 mg/l dissolved oxygen and producing about 2 moles of H^+ for each mole of ammonia N oxidised. From the equation:

$$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + 2H^+ + H_2O \qquad 2.12$$

and $$H^+ + HCO_3^- \rightarrow CO_2 + H_2O \qquad 2.13$$

approximately 7.13 mg of bicarbonate alkalinity as $CaCO_3$ would be required to neutralise the H^+ ions produced during oxidation of 1.0 mg of ammonia N. It is further reported that the rate of conversion of ammonia N to nitrite N essentially controlles the rate of overall nitrification.

Adams et al. (1977) reported that nitrifying organisms exhibit very slow growth in comparison to the heterotrophic organisms. In addition to that they are very sensitive to environmental conditions such as pH, temperature, dissolved oxygen concentration and organic substances. Nitrifiers require inorganic carbon sources such as carbon dioxide, bicarbonates or carbonates. Haug et al. (1972) observed that nitrification is efficient at pH 7.8-8.5 at temperatures of 28-32°C and at dissolved oxygen level above 3.0 mg/l.

Anthonisen et al. (1976) found that both free ammonia N (NH_3) and free nitrous acid (HNO_2) inhibit nitrifying organisms. They quoted Warrington having observed that nitrification proceeds more rapidly in culture placed in the dark than on open bench. Hooper et al., as quoted by Anthonisen et al. (1976), observed that complete inhibition of Nitrosomonas activity occures at 420 lux. Anthonisen et al. (1976) reported that organic matter does have a depressing effect on nitrification. The inhibitory effect of organic matter is reported to be due to dissolved oxygen limitations.

In a study on the oxidation of concentrated ammonia wastewater generated in a fertiliser complex, Hutton et al. (1975) found that efficient removal of ammonia N was achieved at 30°C and pH of 8.4-8.6. They could not maintain an MLVSS concentration of more than 300-500 mg/l. At detention times longer than 10 days the ammonia N removal rate was 0.6 mg/day/mg MLVSS. Removal rates

greater than 90 percent were achieved at retention times longer than 30 days. The raw waste contained 725 mg/l ammonia N and 1000 mg/l alkalinity at pH of 8.0-8.4. The effluent quality after nitrification was 75 mg/l ammonia N, 75 mg/l nitrate N, 575 mg/l nitrite N and 70 mg/l suspended solids.

Das et al. (1965) observed that 40 hours of aeration was required for the nitrification of 1000-2000 mg/l of ammonia N. However nitrification was more efficient in 20 hours when the influent ammonia N concentration was restricted to 500 mg/l. Siddiqui et al. (1971) stated that the ammonia N concentration of 500 mg/l in fertiliser wastes could be brought down to 50 mg/l by nitrification. Hing et al. (1976) observed that the alkalinity required to nitrify sludge lagoons effluent having 780 mg/l ammonia N is approximately 5600 mg/l as $CaCO_3$. Rotating disc system was used with sodium carbonate to supplement alkalinity. Ammonia N removals in excess of 99 percent were achieved at a detention time of 1.5 days and a surface loading of 2.9 g/day/cm^2 at 22-26°C.

2.8.9.2. Biochemical Denitrification: McCarty et al. (1969) established a close relationship between predicted and observed removals of COD and the oxidised nitrogen. The stoichiometric relationship developed by them is as follows:

$$C_m = 2.47\ NO_3^-N + 1.53\ NO_2^-N + 0.87\ DO \qquad 2.14$$

where, C_m = Methanol requirement

and all units being in mg/l of respective compounds.

Stensel et al. (1973) obtained the methanol requirement as follows:

$$C_m = 2.3(NO_3^-N_{inf} - NO_3^-N_{eff}) + 1.0\ DO \qquad 2.15$$

Further McCarty et al. (1969) proposed the following equation for the overall nitrate removal:

$$NO_3^- + 1.08CH_3OH + H^+ \rightarrow 0.065C_5H_7O_2N + 0.47N_2 + 0.76CO_2 + 2.44H_2O \qquad 2.16$$

From this equation the quantity of methanol required for denitrification can be calculated. From Equation 2.14 it can be seen that 1000 mg/l of COD is needed to denitrify 270 mg/l nitrate N or 435 mg/l nitrate N assuming no dissolved oxygen in the system. The optimum pH was in the range of 6.0-8.0. The gaseous end products are reported to be nitrous oxide, nitrogen and nitrogen dioxide.

Das et al. (1965) reported that 0.2 percent of sucrose is required for the denitrification of nitrified fertiliser effluents. The efficiency at 30 hours of detention period was 90 percent. Shah et al. (1978) reported that even very low dissolved oxygen concentration of 0.2-0.4 mg/l inhibits denitrification reaction. St. Amant et al. (1969) documented that 90 percent reduction of nitrate N was obtained at 1.0 hour detention in sand columns. Francis et al. (1977) found that 0.6 mg of methanol is required

to denitrify 1.0 mg of nitrate N. Shah et al. (1978) reported that the growth rate of denitrifying organisms is 6.8 day^{-1}.

2.8.10. Algal Pond Treatment:

The capability of algae in extracting nutrients from the ecosystem is made use of in the algal pond process. Viewed in terms of nutritional requirements and harvesting facilities pond treatment has been found to offer the easily exploited biological system. Bhattacharrya et al. (1966) observed that the pond treatment is feasible for treating high nitrogenous wastes. With an initial ammonia N concentration of 116 mg/l the algal pond was able to reduce the same to 12 mg/l in a period of 6 days. No inhibitory effect was observed up to an ammonia N concentration of 500 mg/l at pH 6.0-6.6. Algal growth and ammonia N utilisation were enhanced when 20 percent of carbon dioxide was mixed with the air. However when glucose was used as carbon source better flocculation, higher growth rates and improved ammonia N removals were obtained. It is reported that harvesting is easy at pH 4.0-4.2.

Chlorella pyrenoidosa, Chlorella ellipsoidea and Chlorella vulgaris were the main species. Average ammonia N removal rate was one tenth of the rate of algal growth at the optimum pH of 6.0-6.6. In their later studies, Bhattacharrya et al. (1968) observed the algal growth as 240-320 mg/l/day at a pH range of 6.0-6.6. 8 percent carbon dioxide was bubbled with air. Golueke et al. (1967) reported that when ammonia N exceeds 45 mg/l algal

production is reduced considerably. They further observed that species Chlorella and Scenedesmus were alternating with seasons.

Bhattacharrya (1969) stated that algal culture in urea plant effluents in shallow outdoor tanks is a promising method for the removal of urea N, provided other nutrients are supplemented. Further it is reported that Chlorella pyrenoidosa is not affected by ammonia N up to a concentration of 1100 mg/l and urea N up to 5000 mg/l, the pH being maintained at 6.0-6.6 by bubbling carbon dioxide enriched air. The algae growth was found to be 150-200 mg/l/day corresponding to 15-20 mg/l/day urea N removal. It is also shown that 1.0 g of nitrogen requires 200-250 l of 10 percent carbon dioxide enriched air.

2.8.11. Enzyme Process:

Smit et al. (1976) devised a method of converting urea N in effluent steams by urease enzyme. At a pH of 9.2 and temperature of 28-37°C Bacillus pasturii was cultured on fixed bed of carbon and nutrients and oxygen were supplied. Urea was degraded to ammonia and carbon dioxide. It is reported that long duration required for achieving optimum column performance, sensitivity of the microorganism to feed fluctuation and constant supply of air are the drawbacks of this technology.

3. ENVIRONMENTAL EFFECTS ON GROWTH OF ALGAE

3.1. Photosynthesis in Algae:

It is not contemplated to go into the complete details of algal photosynthesis in this thesis. However essential features that are more appropriate from the point of view of this work are mentioned. The solar energy that falls on earth is trapped by the primary producers. They synthesise carbohydrates from atmospheric carbon dioxide or natural alkalinity of water. McGriff (1970) quoted Tamiya having gone on record that the complete physiological process can be considered as photosynthesis and formative metabolism.

Formative metabolism comprises of a series of biochemical and physiological processes except photosynthesis, such as nitrogen metabolism and synthesis of organic components and structural

elements of cells, culminating in the formation of autospores and their ripening into vegetative cells. Photosynthesis is defined as a light dependent reaction involving two phases, namely light and dark which correspond to photochemical and biochemical reactions respectively. According to Arnon (1960) photosynthesis comprises of both cyclic and noncyclic photophosphorylation which account for the conversion of light energy into chemical energy and its subsequent use by the cell. ATP and NADH are used for the formation of sugar and starch from carbon dioxide.

Calvin (1962) elucidated the second phase, namely the dark phase of photosynthesis. This is called the carbon reduction cycle. In this cycle the carbon dioxide molecule reacts first with ribulose diphosphate to form two molecules of 3-phosphoglyceric acid. Other compounds are related in a cyclical series of reaction, called Calvin's cycle which serves to regenerate the carbon dioxide acceptor molecule and to synthesise hexose sugars.

3.2. Factors Affecting the Growth of Algae:

Growth of algae is governed by many factors. The most important ones are: light intensity, temperature, carbon dioxide concentration, macronutrients, micronutrients, turbulence, autoinhibitors and characteristics of the organisms.

3.2.1. Light Intensity:

The compensation point is defined as the light intensity at which photosynthesis is balanced by respiration so that the net gas exchange and hence the net growth of algae is zero. Only that portion of the light in excess of the compensation point is available for growth. Rabinowitch (1951) reported that the compensation light intensity for Chlorella is 400 lux at 25°C. The compensation point will shift for various conditions since it is mainly dependent upon respiration and the optical density. Siddiqui et al. (1972) quoted Oswald having stated that the compensation point is reached around 210 lux.

Saturation light intensity is defined by Rabinowitch (1951) as the intensity at which saturation begins when the most exposed chlorophyll molecules receive certain light flux and become complete and no more light is utilised when the most deeply shaded molecules obtain this light intensity. Saturation point depends on temperature and algal species. Krauss (1956) reported that the saturation intensity for Chlorella cultures is 6400 lux. Gates et al. (1964) found the value to be 7200 lux for a mixed culture containing Chlorella and Scenedesmus. Mayer et al. (1964) documented that Chlamydamonas and Chlorella pyrenoidosa become light saturated only at 21000 lux.

Inhibitory point is one where a reduction in photosynthesis is felt. McGriff (1970) quoted Tamiya having fround the inhibitory intensity for Chlorella as 2000 lux at 7°C. The above value.

at 15°C is 25000 lux and at 25°C it is 50000 lux. Krauss (1956) stated that any energy in excess of 10 kilo lux is not used in photosynthesis. Efficiency of photosynthesis is not only dependent upon wavelength and intensity of light source but also upon the ratio of light and dark periods. In a dense algal culture the light and dark cycles can be provided by mixing.

Hermann et al. (1958) derived an expression to find the depth to which light of various intensities can penetrate into a pond. The Briggsian form of the Beer-Lambert law is reported as:

$$\frac{I_o}{I} = 10^{kcd} \quad \text{or} \quad \log \frac{I_o}{I} = kcd. \qquad 3.1$$

where, I_o = Original intensity of radiation

I = Intensity subsequent to penetration

k = Absorption coefficient

c = Concentration of the absorbing material, (g/l)

d = Depth or length of light path (cm).

Now

$$D = \log \frac{I_o}{I} \qquad 3.2$$

where, D = Optical density.

Then

$$D = kcd \qquad 3.3$$

or

$$d = \frac{D}{kc} \qquad 3.4$$

From this expression the depth of penetration of light into any suspension of known concentration could be found out.

Oswald (1963) proposed certain empirical equations for the efficiency of conversion of light energy. The equations are:

$$F = 2.1(5.1 - \ln H) \qquad 3.5$$

and

$$F = 5.6(4.6 - \ln P) \qquad 3.6$$

where, F = Percentage efficiency

H = Average incident light energy on the entire face of growth tubes (cal/l/min)

and P = Time of continuous light duration expressed as the percentage of 24 hours.

3.2.2. Temperature:

Rabinowitch (1956) indicated that the biokinetic range within which plants adapted to moderate climates lies between 0-30°C. The algal growth varies according to the temperature. The detention times of reactors with algal culture vary according to vant Hoff-Arrhenius equation:

$$\frac{t}{t_o} = 1.072^{(T_o - T)} \qquad 3.7$$

where, t = Reaction time required at temperature T°C

and t_o = Reaction time required at temperature T_o°C.

The effect of temperature on photosynthesis is influenced by carbon dioxide concentration and light intensity. It is documented that within the range of 4-16°C the algal growth is independent of temperature. When light intensity is high and carbon dioxide concentration not limiting, photosynthetic efficiency is determined by the rate of carbon dioxide diffusion inside the cell. Lewin (1962) stated that few common fresh water algae grow beyond the temperature range of 25-30°C. He indicated that the optimum temperature for the growth of Chlorella pyrenoidosa is 25-26°C. Sorokin (1960) and Myers (1953) had gone on record that the growth of Chlorella pyrenoidosa is higher at 25°C than that at 20°C or 30°C at light saturation. According to Gates et al. (1964) the growth rate of mixed algal culture at 30°C is higher than that at lower temperatures. Bartsch (1961) reported that growth of Chlorella and other algae reachs maximum rate at 25-30°C.

Davis et al. (1953) had reported that night temperature influences the growth of algae. The greatest growth occurs with a night temperature of 15°C and declines above and below this value. Night temperatures of 5, 30 and 35°C are definitely more unfavourable. Even with cooler night temperatures, 35°C day is too warm for production of more yield.

Round (1966) reported that at high light intensities increase in temperature would raise the light saturation point. He further stated that high temperature strains of Chlorella tolerate

32100 lux at 39°C whilst at 25°C photosynthesis is inhibited by intensity above 10700 lux. Bartsch (1961) quoted many authorities to state that exceptionally good growth of some strains of Chlorella is observed at 39°C. Oswald (1963) has given the empirical formula for photosynthesis as temperature dependent as:

$$F = 3.48(\ln T - 1.85) \qquad 3.8$$

where, F = Percentage efficiency

and T = Temperature (°C).

3.2.3. Carbon Dioxide Concentration:

The exact quantity of carbon dioxide required for photosynthesis depends on various factors such as light saturation, temperature and the algal species. McGriff (1970) reported that 0.03 percent carbon dioxide is sufficient to sustain optimum photosynthesis in algae. He quoted Emerson et al. having obtained the carbon dioxide concentration as 0.1-0.5 percent for optimum photosynthesis.

According to Round (1960), air enriched with 5 percent carbon dioxide is quite suitable for culture of algae. Myers (1953) had gone on record to state that the growth of algae is independent of carbon dioxide concentration when it is present in 0.1-5.0 percent. Oswald (1963) maintained that carbon dioxide concentration of 0.03-2.0 percent in air is found to have direct effect on photosynthetic growth of Euglena gracilis. Beyond

2.0 percent carbon dioxide the efficiency declines. Studies of Golueke et al. (1959) on Chlorella and Scenedesmus revealed that maximum photosynthetic efficiency was obtained at carbon dioxide concentration of 1.0-2.0 percent. Oswald's (1963) empirical formula for carbon dioxide concentration is given as follows:

$$F = k P_c^{0.5} \qquad 3.9$$

where, F = Percentage efficiency

k = 1.0

and P_c = Percentage carbon dioxide in air.

3.2.4. Macronutrients:

Besides carbon dioxide the macronutrients essential for algal growth are nitrogen and phosphorus.

3.2.4.1. Nitrogen: Algae are capable of assimilating organic, ammonia, nitrate, nitrite and elemental nitrogen depending on the species. It has been reported by Lewin (1972) that the efficiency of glucose utilisation for cell synthesis in Chlorella pyrenoidosa is 16 percent more with ammonium N than with nitrate N and the efficiency of light conversion is about 30 percent greater. And it was observed that at low light intensity cell synthesis was faster from ammonium N than from nitrate N. Ammonium N is often preferentially taken when it is supplied together with nitrate N in Chlorella. Nitrite N in low concentration had been found to serve as a source of nitrogen.

Round (1966) quoted many authorities having observed that Chlorella cultures grown in solution containing glucose and nitrate N gave off oxygen even in the absence of carbon dioxide. When carbon dioxide is bubbled the oxygen evolution is not increased. This suggests that nitrate N is acting as an alternate hydrogen acceptor in photosynthesis. When organic nitrogen is supplied, the compounds are deaminated and then only taken up. It had been further emphasised that whatever the form of nitrogen it is only in the form of ammonia that the nitrogen is accepted in the metabolic pathways. It had been further reported that the normal requirement of nitrogen in cultures of Chlorophycea is found to be 6.5-8.3 percent of ash free dry weight.

Round (1966) quoted Manten having obtained the result that excessive absorption of nitrogen could be induced in algae if they are grown in solutions deficient in manganese, boron and zinc. Lewin (1972) had documented that urea could serve as sole nitrogen source for Chlamydamonas, Chlorella and other unicellular green algae. He proposed that urea could serve as the best nitrogen source for mass cultures of Chlorella, as the growth is faster than that in nitrate and high concentration of urea is not toxic.

He quoted Hattori having suggested that urea is taken up by Chlorella pyrenoidosa and Chlorella ellipsodiea without preliminary breakdown to ammonia. It was found that during

urea assimilation the carbon molecule was released as carbon dioxide while amide groups combine with an acceptor with no free ammonia being formed. Hattori (1958) found that when ammonium sulphate was added to nitrogen starved cells of Chlorella, 60 percent of assimilated ammonia was in soluble form and the rest in insoluble form. When urea is assimilated no trace of urea or ammonia is found in the cells. In the case of urea and ammonia considerable portion is converted into insoluble form.

He proposed the pathways of ammonia and urea as follows:

Ammonia → Amide (glutamine) → Arginine → Cells

Urea → Arginine → Cells

Birdsey et al. (1962) reported that Chlorella pyrenoidosa, Chlorella vulgaris, Scenedesmus obliquus, Chlamydomonas reinhardtii, Chlorella spp. and Scenedesmus spp. are capable of using urea, uric acid, potassium nitrate and ammonium chloride when grown in medium containing 70-100 mg/l N. Chrost et al. (1975) quoted many researchers having found that urease activity could not be traced in algal cells. He further reported that the nitrogen concentration required by Chlorella spp. for fastest cell division is 260-600 mg/l N.

Williams et al. (1977) reported that addition of urea to cultures of Chlamydomonas reinhardtii and Chlorella pyrenoidosa causes an increase in the accumulation of urea amidolyase only if ammonia is not present in the culture. It is urea amidolyase that causes

the adaptive activity for urea hydrolysis. Simultaneous addition of urea and ammonia to a cell culture results in a lack of enzyme accumulation. This is due to the repressing effect of ammonia. Hattori (1957) revealed the order of rapidity of assimilation of various nitrogen sources as:

Ammonia > Arginine > Urea > Nitrate > Ornithine > Citrulline

McCarthy (1971) reported that the presence of nitrate has little effect on ammonia uptake but suppresses urea uptake by 40 percent. The presence of ammonia effectively prevents the uptake of either nitrate or urea. The presence of urea has little effect on ammonia uptake but suppresses nitrate uptake to 40 percent.

3.2.4.2. Phosphorus: Shapario et al. (1965) reported that most of the organismsincluding algae utilise phosphorus that is in the orthophosphate form. Arnon (1960) demonstrated the need for phosphorus in the photosynthetic reactions. Any deficiency in phosphate would affect the vital process of photosynthesis and metabolism. Sawyer et al. (1967) suggested a critical level of 0.01 mg/l P for microorganisms. Round (1962) quoted Kelchim et al. having found that phosphorus requirement of Chlorella to be 2.0-3.0 percent of dry weight. Phosphate deficiency in algae results in the accumulation of fat. Kuhl (1967) maintained that algae normally contain 3.0-5.0 mg/l P per gram of cells. Brar et al. (1975), Maloney (1966) and Sanders(1972) proved experimentally that there exists luxury uptake of phosphorus in

activated sludge and algae. Borchardt et al. (1968) defined luxury uptake as that phosphate incorporated into the cell mass above the critical level or uptake occurring when phosphate in the cell mass exceeds 3.0 percent on the dry weight basis. They obtained 3.0-9.0 percent of phosphorus in the cell mass.

Vacker et al. (1967) found that activated sludge that exhibited high removals of phosphates contained as much as 5.0 percent as phosphorus. Hopson et al. (1973) observed that cultures that are carbon stressed so as to allow only increase in mass and not in cell division do take up excess phosphorus as much as 4.0 percent on the dry weight basis. Gerloff (1969) reported that plants will absorb an element in concentrations far above the critical level, but this luxury consumption is not associated with increases in yield. So sufficient amount of phosphate should be provided for the proper metabolism and photosynthesis of algae and bacteria.

3.2.5. Micronutrients:

Micronutrients are those elements which constitute part of enzyme system and are required in trace amount by microorganisms such as algae and bacteria for their growth and reproduction. Of these iron, manganese, molybdenum, zinc, copper and vanadium are of importance. Myers (1953) quoted Krauss having established that the uptake of nitrate from the culture medium by Scenedesmus is more rapid in the case where micronutrients such as zinc, boron

and manganese are absent than from the ones where these are present. This results in high nitrogen content per cell.

3.2.5.1. Iron: Iron is a constituent of many enzyme and cytochromes and so iron deficiency will result in retarded growth. Lewin (1972) had documented that a concentration of 1.8×10^{-7} to 2.6×10^{-8} M is found adequate for normal growth of Chlorella. He quoted Walker having found that 1.0 gram dry weight of Chlorella pyrenoidosa contains 30 g of iron. McGriff (1970) quoted Eysler having indicated that a minimum level of 1.8×10^{-5} M of iron is required to obtain maximum growth of Chlorella pyrenoidosa. Normally surface waters contain iron at a concentration of 0.50 mg/l whereas ground waters may contain as much as 50 mg/l at pH 5.0-8.0.

3.2.5.2. Manganese: Manganese is essential for the autotrophic growth of algae. However according to Lewin (1972) Scenedesmus does not depend on manganese for photosynthesis. The critical level for autotrophic growth of Chlorella pyrenoidosa is found to be 1.0×10^{-7} M whereas for heterotrophic growth 1.0×10^{-9} M alone is needed. The concentration of manganese in natural water is of the order of 0.05-0.22 mg/l (McGriff, 1970). In ground waters it seldom exceeds 1.0 mg/l.

3.2.5.3. Calcium and Magnesium: Calcium ions play an important role in the maintenance of cytoplasmic membranes and wall structures. Krauss (1953) reported that the calcium requirement for Chlorophyta is 0.2-19.0 mg/l. Magnesium, being a constituent

of chlorophyll, is an essential element in algae. Round (1966) maintained that if calcium and magnesium are present in large quantities, algae are tolerant to wide ranges of calcium/ magnesium ratios. Magnesium deficiency interrupts in the cell division of Chlorella and Ankistrodesmus. This results in enlargement of cells. Krauss (1953) quoted Chu having observed that magnesium requirement for Chlorophyta ranges from 2.0-7.0 ppm.

3.2.5.4. Other Trace Elements: Table 6 as reported by Eysler (1967) gives the basic requirements of some of the trace elements and micronutrients required by algae.

3.2.6 Autoinhibitions:

The effect of autoinhibition on algal growth is still a controversial subject. Myers (1953) quoted Pratt having demonstrated that an inhibitor called Chlorellin excreted in small amounts by Chlorella vulgaris has a self inhibiting effect. However the composition was not revealed and the postulation has not obtained wide acceptance. Myers (1954) established that there is no evidence to prove the Chlorellin theory of inhibition. He added that any inhibition that is likely is due to growth limiting conditions such as light intensity and depletion of nutrients.

Table 6 - Micro-inorganic Elemental Requirements for Algae

Element	Optimal Concentration		Cellular Function	Known Enzymes with Element
	Without Chelate ppm	With EDTA ppm		
Iron (Fe)	0.05	5.0	Photosynthesis, Respiration	Peroxidase, Catalase, Cytochromes, Cytochrome oxidase, Ferrodoxin for photosynthesis
Manganese (Mn)	0.005	5.0	Hill reaction, Krebs cycle	
Zinc (Zn)	0.001	6.0	Hydrogen transfer both in respiration and photosynthesis	Carbonic anhydrase, Carboxy peptidase and other specific dehydrogenases
Copper (Cu)	0.001	0.4	Respiration and photosynthesis	Lactase, Ascorbic acid oxidase, Polyphenol oxidase, Plastocynin for photosynthesis
Cobalt (Co)	0.00004		Nitrogen fixation	
Molybdenum (Mo)	0.00001 0.01		Reduce nitrate, Nitrogen fixation	Nitrate reductase
Boron (B)	0.1		Nitrogen fixation	
Vanadium (V)	0.1		Photosynthesis	
Calcium (Ca)	0.05 0.3	40.0	Digestion, Nitrogen fixation	Amylases
Sodium (Na)	2.0		Required by blue-green algae	

3.2.7. Characteristics of the Organisms:

Growth is affected by the same set of conditions in different ways for different species. Myers (1953) demonstrated that the specific growth rate for Chlorella pyrenoidosa is 1.96 day^{-1} at 25°C. Thus 1.0 mg dry weight of cells containing 0.50 mg of carbon increases to 1.082 mg dry weight and 0.54 mg of carbon after steady state growth for one hour at maximum rate. It was also shown that cells grown at 5400 lux have about twice as large size as compared to those grown at 1100 lux. But chlorophyll content remains the same. The large cells are restricted in cell division but not in size. Bristol et al. as quoted by Myers (1953) also came to the same conclusions.

At low intensity of light the specific growth rates of Scenesdesmus costalatus is found to be:

(i) Light + carbon dioxide = 0.35 day^{-1}

(ii) Dark + glucose = 0.48 day^{-1}

(iii) Light + glucose = 0.87 day^{-1}

However at higher light intensities the specific growth rate is reported to be 1.08 day^{-1} under all the conditions stated above. So it was concluded that at light saturation, the growth rate is not limited by rate of carbon assimilation but by some other process.

Zajic et al. (1970) quoted Griffiths having demonstrated that Chlorella vulgaris cells increase very much in size during the

lag phase and can be induced by glucose addition. It is further reported that cells of the same strains of algae are much larger in heterotrophic and mixotrophic cultures than in autotrophic cultures. This is an advantage from the point of view of flocculation and nitrogen removal.

Oswald et al. (1953) are of the opinion that at longer detention periods a large percentage of cells cease to multiply and enter a phase of cell enlargement. Gotaas et al. (1951, 1953) reported that cells of Euglena gracilis become enlarged in size, reproduce slowly and settle rapidly. Pipes (1961) reported that carbon is most often the limiting factor in the growth of algae in high rate stabilisation ponds. This causes the enlargement of cells.

3.2.8. Turbulence:

Several investigators have shown that turbulence plays an important role in increasing algal growth through efficient utilisation of light. Algae growing fast would attenuate light by mutual shading. As a result growth rate is slowed down. This effect could be alleviated either by using low density cultures or turbulence in high density cultures. It has been shown by Myers (1953) and Bartsch (1961) that the dark period should be ten times the light period to obtain the maximum growth of algal cells grown in sunlight at 25°C. McGriff (1970) quoted Olinger having concluded that induced turbulence enables more efficient utilisation of nutrients by mixed culture of

algae. He quoted David having obtained 70-300 percent increase in algal yield by inducing turbulence. It is further reported that the response of Chlorella to a growth substance can be increased and prolonged by agitating the growing culture. Moreover agitating facilities could release the autospores from the mother cells thereby exposing them to the growth substances for quick assimilation.

Bartsch (1961) reported that under idealised condition of high turbulence at full sunshine, increase in photosynthesis and oxygen evolution was found to reach seven fold. Gates et al. (1964) quoted many investigators having shown that photosynthesis reaction is time-light intensity dependent. Time-intensity relationship indicates that with sufficiently high intensity and proper adjustment of light and dark periods a growth rate can be attained which is equivalent to the growth rate under continuous exposure at saturation light intensity. This objective can be achieved by agitating high density cultures.

3.2.9. pH:

Bush et al. (1963) reported that most algae flourish at a pH range of 7.0-8.5, with higher and lower values leading to growth inhibition. Wilt et al. (1960) observed that good growth of algae such Chlorella, Scenedesmus and Ankistrodesmus occurs in the pH range of 6.0-9.5 with best results at pH 8.3. Pipes et al. (1961) maintained that most heterotrophic organisms

present in a pond have a pH tolerance range between 6.0-9.0 with optimum value lying at 7.0-8.0. Although algae have wide pH tolerance range than bacteria, high pH ranges are known to inhibit photosynthetic oxygen production. It is further reported that at a pH of 8.0,BOD reduction increases while at lower ranges the same decreases.

Oswald et al. (1957) inferred that high pH values above 9.8 do limit bacterial growth. Alagarswamy (1967) observed that a pH range of 6.2-9.5 is favourable for algal growth. Oswald (1960) reported that the bacterial activity is inhibited above pH values of 8.0 unless acclimatisation is done. When excess ammonia and carbon dioxide are assimilated growth slows down. It is further reported that the effect of pH on bacteriostasis and ammonia loss is pronounced at higher temperatures. Gates et al. (1964) maintained that Scenedesmus is capable of keeping constant growth in the pH range of 5.5-11.0. Kerr et al. (1972) observed that population of bacteria was lower below pH 5.5 and above 8.0 and total inhibition took place above pH 9.0. Chaudhuri et al. (1969) are of the opinion that growth of Chlorella is enhanced between pH 6.5-9.0 whereas that of Scenedesmus is excellent in the pH range 6.5-7.5.

3.3. Mixotrophic Growth of Algae:

Classically all algae form their protoplasm solely from carbon dioxide by photosynthesis. However some are facultative

heterotrophs and are able to utilise organic substances as a source of carbon. Zajic et al (1970) quoted many investigators having established that heterotrophic growth is accelerated upon introduction of light. This type of growth is called photo-heterotrophy or mixotrophy, contrasted to photoautotrophy in which cellular material is synthesised solely from inorganic carbon in the light.

Zajic et al. (1970) reported that Chlorella, Scenedesmus, Chlamydamonas, Ankistrodesmus, Chlorococcum, Pictyosphaerium are the main species that can be grown in a mixotrophic way. They quoted Dvorakova et al. having grown Chlorella pyrenoidosa and Scenedesmus obliquus in a medium with 0.05 M sugars such as fructose, galactose, glucose, maltose, cellobiose, sucrose and soluble starch. With light saturation at 2000-10000 lux and with 2.0 mg of glucose, 1.0 mg of cellular material of Chlorella was obtained. However Scenedesmus needed only 1.26-1.51 mg of glucose to build 1.0 mg of cellular material. In the dark 3.0 mg of glucose was needed for building up of 1.0 mg of cellular material in either species. It is further reported that the total mixotrophic growth is greater than the sum of the rates due to either photoautotrophy or heterotrophy in the dark.

3.4. Algal Excretional Products:

Wood (1968) reported quoting Wetzel, that up to 10 percent of the assimilated products may be excreted as glucose during active photosynthesis. Stanier et al. (1974) have documented that

algae adapted to symbiotic environments facilitate the unidirectional transport of carbohydrates from the algal cell to the non-photosynthetic symbiont. These symbiotic algae excrete much greater proportion of their fixed carbon than do the free living ones. It is the symbiotic association that stimulates profuse transfer of carbohydrates synthesised. It is further reported that the excreted carbohydrate is usually different from the major intracellular carbohydrates of algae. In most cases it is a carbohydrate which the non-photosynthetic partner but not algae themselves can utilise. This is true in most of symbiotic associations. In the algal bacterial symbiosis, a part of organic carbon added may be utilised by algae as well in a mixtotrophic way. However the algal excretion of carbohydrates will make up the deficiency created by algal assimilation.

Mcfeters et al. (1978) reported that symbiotic communities of algae and bacteria are ecologically important in nutrient recycling. Various bacteria have been found in mass cultures of Chlorella. It is further revealed that some bacteria grow well utilising the algal excretions. Sanders (1972) reported that 70 percent of the inorganic carbon fixed by Ankistrodesmus braunii was excreted as organic carbon by-products during a cell cycle. It was noted that the excretion depended on physiological state of cells and was varying throughout the cell cycle. Pipes (1961) observed that algae excrete relatively large amounts

of soluble organic matter if they are growing slowly as they would in oxidation ponds with long detention times.

There are numerous statements in the literature that algae may provide an ecologically important source of organic molecules for the growth or maintenance of heterotrophic bacteria. It has also been demonstrated that organic compounds excreted by algae serve as a source of bacterial nutrients. Mcfeters et al. (1973) further reported that different bacteria including mixed natural population from Surprise lake outlet stream were capable of significant growth in the presence of Chlorella extracellular products. It was observed that the bacteria took up approximately 10 percent of the algal excretion concomitant with active reproduction. These authors quoted Hellebust having documented that some phytoplankton are capable of excreting 25 percent of their photo assimilated carbon during their log growth phase.

Wright et al. have been quoted as having suggested that the algae would require one to three orders of magnitudes greater concentration of organic compounds to metabolise heterotrophically than do bacteria. Therefore symbiotic bacteria closely associated with algae function as a sink for such solutes and prevent algal utilisation of these solutes. However, in most natural habitats bacteria are under carbon starvation due to limited quantity of glycolates excreted by algae compared to bacterial numbers.

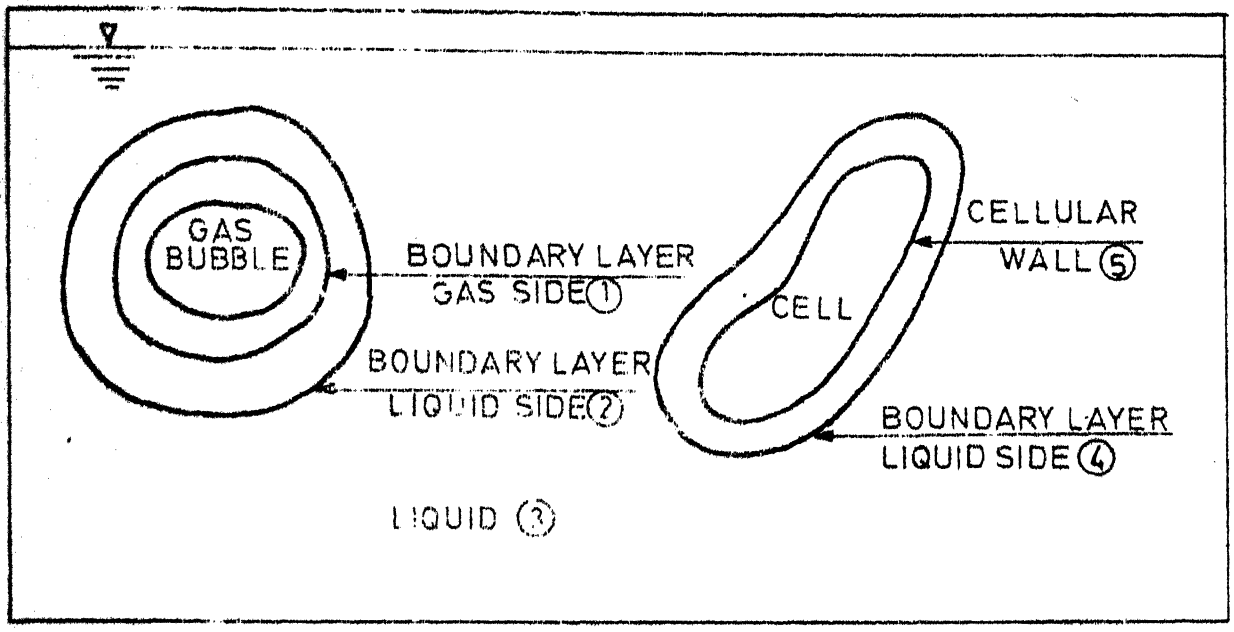
3.5. Carbonate Equilibria in Algal Cultures:

Presently the fluctuation in carbon dioxide in water due to algal growth and other factors will be dealt with. Markl (1978) reported the views of Wordan et al. that the carbon uptake by the cell is most probably in the form of aqueous carbon dioxide (CO_2) and not in the form of carbonate ($CO_3^{=}$) or bicarbonate ion (HCO_3^{-}). The carbon dioxide transport from the gas bubble to the cell is characterised through a series of transport resistances as indicated in Figure 6. Under conditions of normal dense culture of microorganisms the resistance at the gas bubbles is larger than that at the boundary layer of the cell.

Carbon dioxide is a very small component of the normal dry atmosphere occurring to the extent of 0.0314 percent by volume. Consequently ultra-pure water in equilibrium with atmosphere would contain only a very low level of carbon dioxide. However the formation of HCO_3^{-} and $CO_3^{=}$ greatly increases the solubility of CO_2. A large share of carbon dioxide found in water is produced by the metabolic processes of microorganisms. Although CO_2 in water is often represented as H_2CO_3, the equilibrium constant for the reaction:

$$CO_2\ (aq) + H_2O \rightleftharpoons H_2CO_3 \qquad 3.10$$

is only 3.4×10^{-2} at 25°C and only a small fraction of the dissolved CO_2 is actually present as the species H_2CO_3. The CO_2-HCO_3^{-}-$CO_3^{=}$ system in water has been described by Manahan (1974) as follows:



FIG·6 CO_2 TRANSPORT MECHANISM IN TO A CELL

$$CO_2 + H_2O \rightleftharpoons H^+ + HCO_3^- \qquad 3.11$$

$$K_1 = \frac{[H^+][HCO_3^-]}{[CO_2]} = 4.5 \times 10^{-7} \qquad 3.12$$

and $pK_1 = 6.35$

$$HCO_3^- \rightleftharpoons H^+ + CO_3^{=} \qquad 3.13$$

$$K_2 = \frac{[H^+][CO_3^{=}]}{[HCO_3^-]} = 4.7 \times 10^{-11} \qquad 3.14$$

and $pK_2 = 10.33$

From the known values of K_1 and K_2 the distribution of the species could be found from the following equations:

$$a_{CO_2} = \frac{[H^+]^2}{H^+ + K_1[H^+] + K_1K_2} \qquad 3.15$$

$$a_{HCO_3^-} = \frac{K_1[H^+]}{[H^+]^2 + K_1[H^+] + K_1K_2} \qquad 3.16$$

$$a_{CO_3^{=}} = \frac{K_1K_2}{[H^+]^2 + K_1[H^+] + K_1K_2} \qquad 3.17$$

The bicarbonate ions in water have two important functions. In the first place they provide the main buffer system for regulating the hydrogen ion concentration in water while secondly they provide the carbon dioxide for photosynthesis. The

dissolved carbon dioxide is very small and it works out to be 0.4 mg/l at 30°C, 0.5 mg/l at 20°C and 1 mg/l at 0°C. The excess CO_2 increases the H^+ concentration sufficiently to dissolve carbonates as follows (Golterman, 1975):

$$CaCO_3 + H_2O + CO_2 \rightleftharpoons Ca^{++} + 2HCO_3^- \qquad 3.18$$

When some CO_2 is removed from the solution by algae during photosynthesis, the H^+ concentration decreases and pH shoots up. This can be seen from the equations given earlier. Figure 7 illustrates the equilibria of CO_2-HCO_3^--$CO_3^=$ in natural waters.

As K_2 is much smaller than K_1, the solution becomes more alkaline as and when CO_2 is used up during photosynthesis. At such occasions the overall reaction is as follows:

$$2HCO_3^- \rightleftharpoons CO_3^= + H_2O + CO_2 \qquad 3.19$$

This reaction will take place until the equilibrium between HCO_3^--$CO_3^=$ and equilibrium CO_2 and pH have reestablished (Golterman, 1975).

$CaCO_3$ plays an important part in the carbonate equilibria. The solubility of $CaCO_3$ can be increased by an extra amount of free CO_2 which is not in equilibrium with water. Golterman (1975) quoted Tillmans *et al.* having given an approximate relationship between dissolved $Ca(HCO_3)_2$ and the amount of free CO_2 necessary to keep that $Ca(HCO_3)_2$ in solution. It is said that this is applicable to waters where pH is lower than 8.3 because the

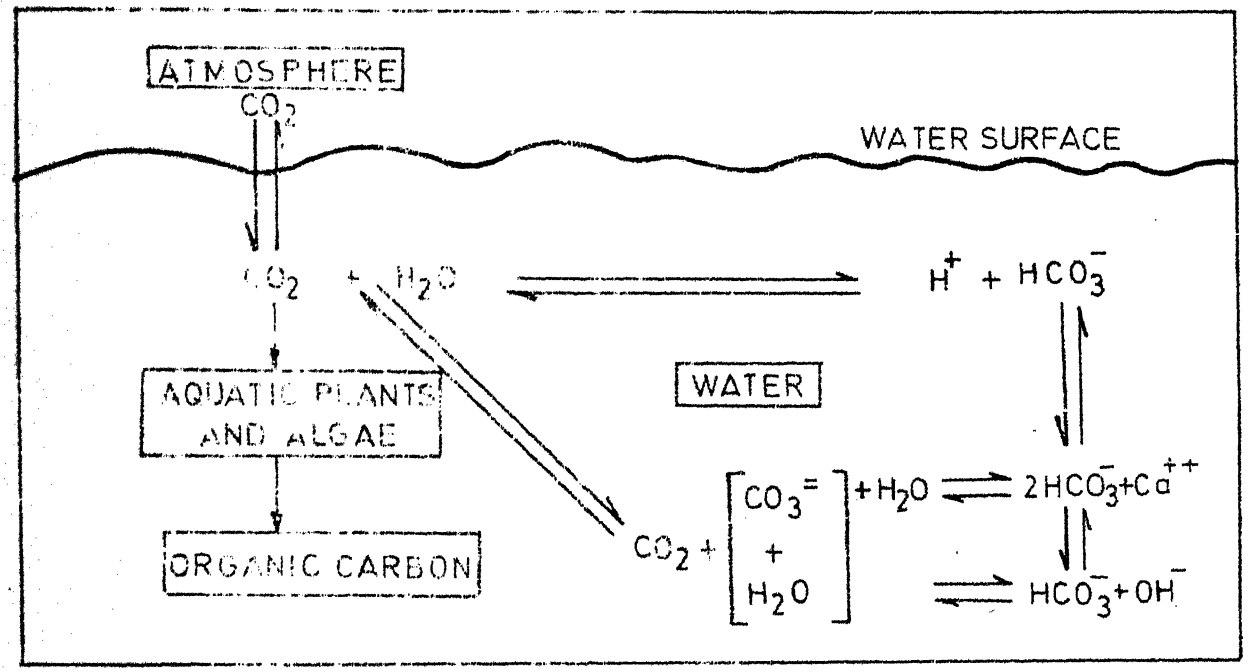


FIG. 7 CARBONDIOXIDE-BICARBONATE-CARBONATE-HYDROXIDE RELATIONSHIP IN NATURAL WATERS

concentration of free CO_2 which could occur is negligible at pH values greater than this. Table 7 illustrates the relationship between $CaCO_3$ concentration and free CO_2.

Table 7 - Relationship between $CaCO_3$ Concentration and Free CO_2

$CaCO_3$ in water	1 meq/l	2 meq/l	3 meq/l	4 meq/l
Equilibrium CO_2	0.6 mg/l	2.5 mg/l	6.5 mg/l	15.9 mg/l

So it follows that if $CaCO_3$ is present, the same can serve as a reservoir for CO_2 while bubbling air or CO_2 in a photosynthesising culture of algae. When the concentration of CO_2 drops the excess $CaCO_3$ will get precipitated.

Goldman et al. (1974) documented that in most natural fresh waters the major pH buffer is the CO_2-H_2CO_3-HCO_3^--$CO_3^=$ system. The total dissolved inorganic carbon available for algae is represented as:

$$C_T = CO_2\ (aq) + H_2CO_3 + HCO_3^- + CO_3^= \qquad 3.20$$

where, C_T = Total dissolved inorganic carbon (moles/l)

$CO_2\ (aq)$ = Aqueous CO_2 concentration (moles/l)

H_2CO_3 = Carbonic acid concentration (moles/l)

HCO_3^- = Bicarbonate concentration (moles/l)

and $CO_3^=$ = Carbonate concentration (moles/l).

Putting CO_2 (aq) + H_2CO_3 = $H_2CO_3^*$. The equation reduces to:

$$C_T = H_2CO_3^* + HCO_3^- + CO_3^= \qquad 3.21$$

The various proportions of CO_2, HCO_3^- and $CO_3^=$ present in water at different pH values are reported by Hutchinson (1957). The values are presented in Table 8.

Table 8 - Proportion of CO_2, HCO_3^- and $CO_3^=$ in Water at Various pH Values

pH	Total Free CO_2	HCO_3^-	$CO_3^=$
4	0.996	0.004	1.25×10^{-9}
5	0.962	0.038	1.20×10^{-7}
6	0.725	0.275	0.9×10^{-5}
7	0.208	0.792	2.6×10^{-4}
8	0.025	0.972	3.2×10^{-3}
9	0.003	0.966	0.031
10	0.0002	0.757	0.243

As stated earlier the utilisation of CO_2 exceeds CO_2 transport rate from atmosphere and so a drain is made in the inorganic $H_2CO_3^*$-HCO_3^--$CO_3^=$ system and a decrease in C_T concentration occurs. The following steps would provide CO_2 to the photosynthetic algae:

$$2HCO_3^- \rightleftharpoons CO_3^= + H_2O + CO_2 \qquad 3.22$$

$$HCO_3^- \rightleftharpoons CO_2 + OH^- \qquad 3.23$$

$$CO_3^= + H_2O \rightleftharpoons CO_2 + 2OH^- \qquad 3.24$$

At pH 8.0-8.5, as HCO_3^- is the major species, it is suggested that CO_2 is extracted from solution according to the reactions given in equations 3.22 and 3.23. However, the reaction given in equation 3.24 is significant only at pH values above 10.0.

When all bicarbonate has been exhausted, $CO_3^=$ is dehydrated and CO_2 is withdrawn raising the pH further. Consequently C_T concentration decreases as the algal growth proceeds, pH rises and the carbonate buffer system is destroyed. However, it had been shown that only a portion of total inorganic carbon could be extracted during intense algal activity in a natural water buffered by $H_2CO_3^*$-HCO_3^--$CO_3^=$ system. For most natural waters approximately one half of the total inorganic carbon could be utilised as CO_2 before the pH rises to 11.0, at which metabolic inhibition is reported to occur.

Considerable research has been done to distinguish between CO_2 (aq) and direct HCO_3^- uptake by various algae. Direct utilisation of HCO_3^-, still a controversial topic, has been described as an ecologically important phenomenon. This is so because the conversion of HCO_3^- to CO_2 (aq) becomes a rate limiting step for the inorganic carbon utilisation.

Similarly, if algae were able to use only CO_2 (aq) as an inorganic carbon source it is possible that the chemical transformation rates of ionic forms of inorganic carbon to CO_2 (aq) could be limiting algal growth. Goldman *et al.* (1974) quoted Kern having pointed out that, whereas the reaction

$$CO_3^{=} + H^+ \rightleftharpoons HCO_3^- \qquad 3.25$$

and

$$HCO_3^- + H^+ \rightleftharpoons H_2CO_3 \qquad 3.26$$

are essentially instantaneous, the dehydration of H_2CO_3 to CO_2 is a relatively slow step:

$$H_2CO_3 \longrightarrow H_2O + CO_2\ (aq) \qquad 3.27$$

where $k_{H_2CO_3} = 26.6/\text{sec}$ at 25°C.

Now it is to be noted that equation 3.22 is the sum total of very rapid reactions depicted in equations 3.25 and 3.26. The direct conversion of HCO_3^- to CO_2 (aq) and OH^-, predominant at pH values greater than 10.0 (equation 3.23) is a much slower reaction than the dehydration of H_2CO_3. Kern concluded that neither dehydration of CO_2 by the reaction:

$$H_2CO_3 \rightarrow CO_2 + H_2O \text{ at pH } 8 \qquad 3.28$$

nor by the reaction

$$HCO_3^- \rightarrow CO_2 + OH^- \text{ at pH } 10 \qquad 3.29$$

is a rate limiting step for algal growth.

King (1970) stated that if algae were unable to utilise the bicarbonate indirectly they would have to rely on available free CO_2 for the photosynthetic carbon sources. He quoted Oswald et al. having suggested that if algae require free CO_2, the dissociation of the bicarbonate would take the form given in equation 3.29. The only difference is that in that case the CO_2 would be released from the bicarbonate ion outside rather than inside the cell. Chaudhuri et al. (1969) documented that 0.1-0.2 percent of sodium carbonate stimulated the growth of algae and also increased the C:N ratio of the cells. They also pointed out that 0.02 percent calcium carbonate was more efficient than sodium bicarbonate.

The resulting hydroxide ion is supposed to be removed by the reaction:

$$OH^- + HCO_3^- \rightleftharpoons CO_3^{=} + H_2O \qquad 3.30$$

King (1970) suggested that the sequence of events is of the following form:

$$HCO_3^- + H^+ \rightleftharpoons H_2CO_3 \rightleftharpoons CO_2 + HOH \qquad 3.31$$

At low pH level the free H^+ ions are got for the reaction given in equation 3.31 but at higher pH levels the necessary H^+ ions would come from dissociation of water. The resulting hydroxyl ion would react with bicarbonate as shown in equation 3.30 and

addition of equations 3.30 and 3.31 would give the following final reaction:

$$2HCO_3^- + H_2O \rightleftharpoons CO_2 + CO_3^= + 2HOH \qquad 3.32$$

If the CO_2 extraction were to continue above pH 10.0, then the reaction would be as follows:

$$CO_3^= + 2HOH \rightleftharpoons CO_2 + HOH + 2OH^- \qquad 3.33$$

In this case the total alkalinity as $CaCO_3$ remains the same although pH rises.

It is to be noted that any material that would serve as a hydrogen donor for the reaction given in equation 3.31 would alter these relationships. Subsequent loss of such materials would cause a decrease in total alkalinity. Any non-carbonate buffer would affect the system to some extent. This would be discussed with relevence to ammonia in the next section.

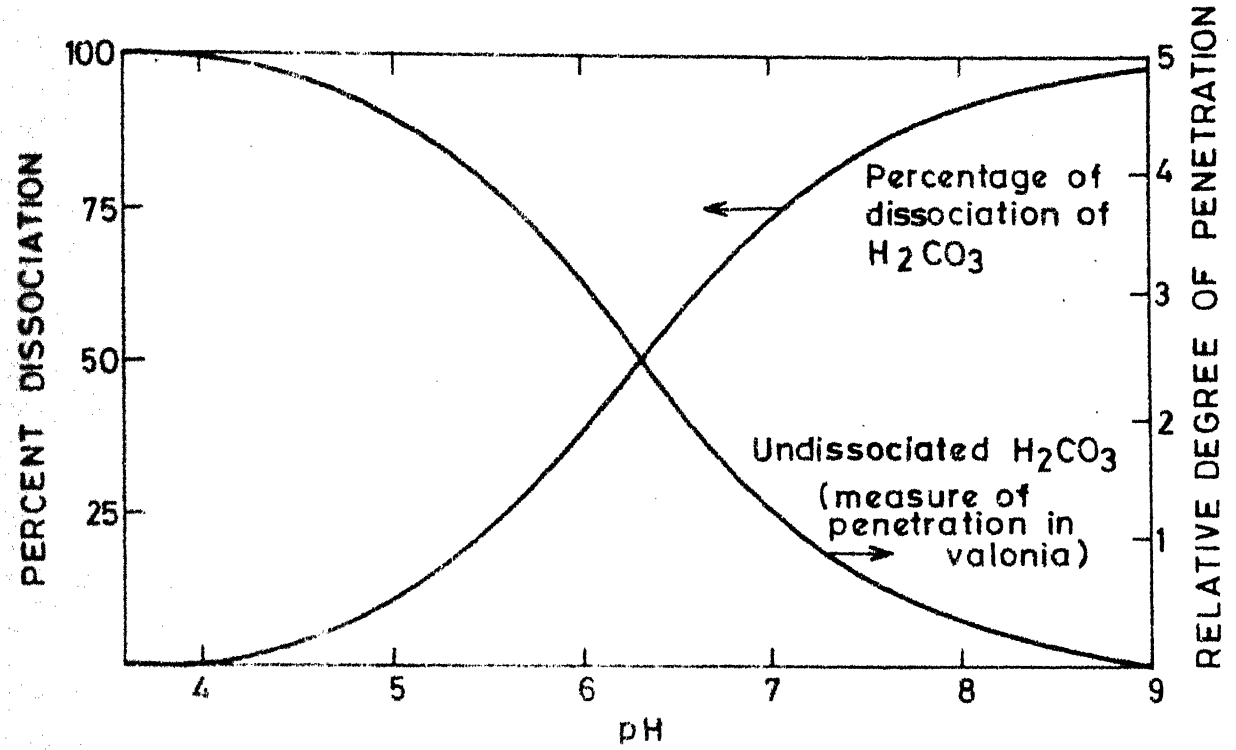
The next most important factor is the penetrability of CO_2 or HCO_3^- into the cells. Classical studies by Collander *et al.* on the entry of substances into the alga *Chara* have been reported by Giese (1973). It was observed that usually molecules with the largest partition coefficient (the ratio of solubility of a compound in oil or fat solvent to its solubility in water) enter the cell more readily regardless of the molecular size. It was also revealed that very large molecules have less chance of entering the cell through the plasma membrane. Sucrose

enters the cells in small amount while substances such as starch and glycogen do not penetrate.

It is indicated that ionisation is yet another factor in the permeability of solutes through plasma membranes. Electrolytes enter the cells more slowly than nonelectrolytes of similar molecular dimensions. Strong electrolytes enter more slowly than weak electrolytes. The degree of ionisation is determined by the pH and by varying the pH within limits safe for the living cell, the penetration also varies.

In Figure 8 the effect of pH on the penetration of carbonic acid in Valonia is shown. At pH 6.34 one half is dissociated. As the pH rises the dissociation increases but the entry of carbonic acid decreases. Conversely with the fall in pH the amount of dissociation decreases, CO_2 is formed and entry of CO_2 into the cell increases. The entry of weak bases follows similar pattern except that in this case the rise in pH suppresses dissociation and enhances penetration. Figure 9 may be referred to.

Giese (1973) quoted Hober having reported that the presence of a charge on an ion decreases its chance of entry into a cell. Ussig (1952) observed that in the case of strong electrolytes monovalent cations such as Na^+ and K^+ enter more readily than divalent cations such as Ca^{++} or Mg^{++} and divalent cations enter more readily than trivalent cations such as Fe^{+++}. Similar is the pattern in the case of anions. It is further elaborated by Giese (1973) that not all ions (cations and anions)



IG. 8 RELATION OF pH AND PENETRATION OF A WEAK ELECTROLYTE (After Osterhout et al. 1926)

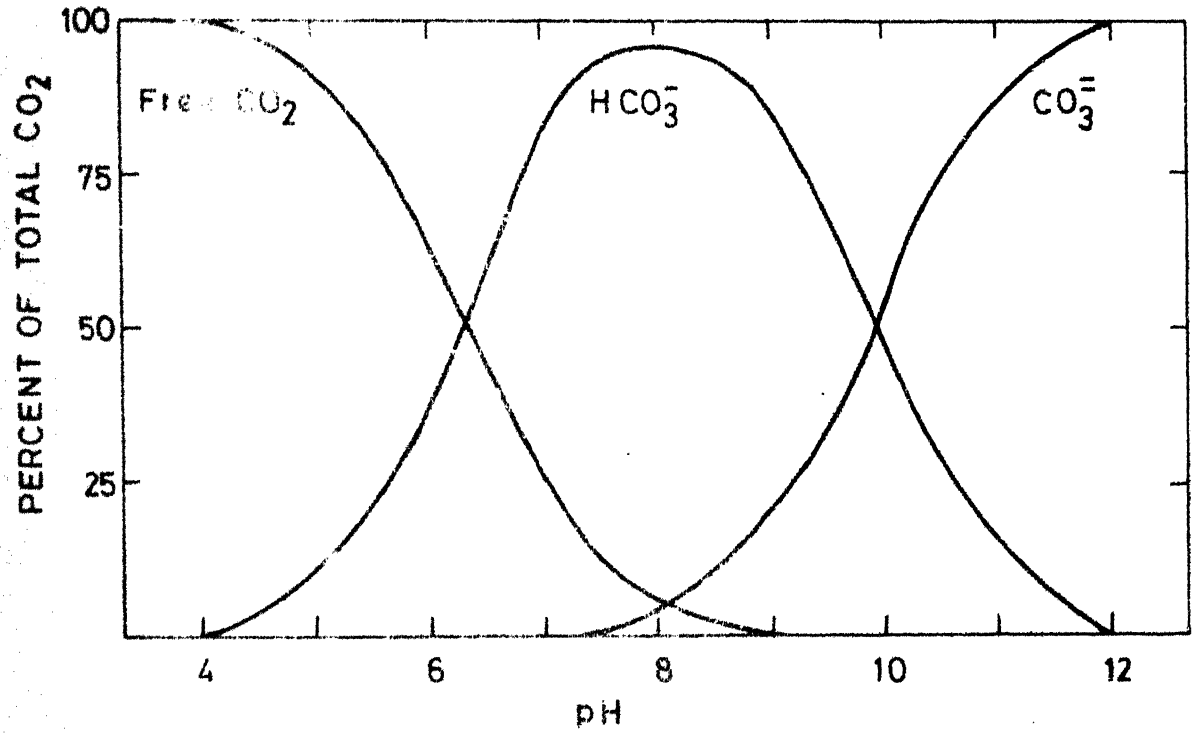


FIG. 9 PROPORTION OF TOTAL CO_2 IN THREE FORMS

of the same valency enter cells at the same rate. For example the ammonium ions enter more readily than potassium ions, potassium ions more rapidly than sodium ions and sodium ions more rapidly than lithium ions. This is explained on the basis of the relative size of hydrated ions, which are much larger than the ions themselves. Thus the permeability to ions is dependent to some extent upon a sieve action of plasma membrane of the cell.

It was revealed that the hydration sphere does not electrically neutralise the ion; on the contrary the residual charge still has its effect upon penetration. The plasma membrane is a mosaic of negatively and positively charged areas. However the overall charge of the plasma membrane is positive.

Hober as quoted by Giese (1973) further revealed that anions enter cells more readily than cations. The passage of cations and anions from an external medium into a cell is in exchange for an ion of like charge inside the cell. Hydrogen ions resulting from metabolic activities are always available for exchange with cations (cation exchange diffusion) and bicarbonate ions of similar origins for exchange with anions (anion exchange diffusion). Some cells are freely permeable to anions while they largely exclude cations. Some others may be quite the other way about.

Natarajan (1970) observed that when ammonia (NH_3) concentration is increased in the culture medium the relative carbon uptake in

marine diatoms is decreased very much. Golterman (1975), Hutchinson (1967) and Gates et al. (1964) reported that while Chlorella pyrenoidosa cannot use HCO_3^-, Scenedesmus quadricauda can utilise the same readily. The inability of most algae to utilise HCO_3^- is due to low permeability or lack of an active transport mechanism to bring the ion into the cell. Kerr et al. (1972) demonstrated that phosphorus, nitrogen and organic carbon regulate the heterotrophic population and inorganic carbon regulates algal population. Giese (1973) reported that calcium ions decrease the permeability of membrane to water and other substances. This is of great significance to cells in any ecosystem.

3.6. Ammonia in Aquatic Environments:

The solubility of ammonia in water is very high. The equilibrium of ammonia with ammonium ion in water exists as:

$$NH_3 + H_3O^+ \rightleftharpoons NH_4^+ + H_2O \qquad 3.34$$

This indicates that the undissociated ammonia in the system depends upon H^+ ion concentration. Applying law of mass action:

$$K_{eq}\ (\text{ammonia}) = \frac{[NH_3][H_3O^+]}{[NH_4^+][H_2O]} \qquad 3.35$$

and

$$2H_2O \rightleftharpoons H_3O^+ + OH^- \qquad 3.36$$

Therefore, $$K_{eq}\ (\text{water}) = \frac{[H_3O^+][OH]}{[H_2O]^2} \qquad 3.37$$

Simplifying equation 3.37, we get

$$K_{eq}\ (\text{water})\ [H_2O]^2 = [H_3O^+]\ [OH^-] = k_w \qquad 3.38$$

and for ammonia the equation is simplified as:

$$K_{eq}\ (\text{ammonia})\ [H_2O] = \frac{[NH_4^+][OH^-]}{[NH_3]} = k_b \qquad 3.39$$

Therefore, $$\frac{k_w}{k_b} = \frac{[NH_3]\ [H_3O^+]}{[NH_4^+]} \qquad 3.40$$

or $$\frac{[NH_4^+]}{[NH_3]} = \frac{k_b}{k_w} \times 10^{-pH} \qquad 3.41$$

where $$pH = -\log_{10}[H_3O^+]$$

Adding 1 to both sides and rearranging the term we get:

$$\frac{[NH_3]}{[NH_3] + [NH_4^+]} = \frac{1}{1 + \frac{k_b}{k_w}\,10^{-pH}} = F \qquad 3.42$$

$$= \frac{NH_3}{(\text{total ammonia concentration})} \quad (\text{Loehr } \underline{\text{et al.}}, 1973)$$

F is the ratio of the undissociated ammonia to the total ammonia concentration and is the fraction of ammonia in the undissociated form. Equation 3.42 can be further rearranged as:

$$\frac{10^{pH}}{10^{pH} + k_b/k_w} = F \qquad 3.43$$

The undissociated ammonia concentration can be obtained by multiplying the total ammonia concentration by $\frac{17}{14}$ F. The equation is dependent on temperature and pH. The values of k_b and k_w increase with temperature. The values of F for specific temperature are given in Appendix A.1. The relationship between F and (1-F)/F and also the relationship between pH and F are given in Appendix A.2.

Coming back to the relationship of dissociation of ammonia, the equation can be put as:

$$NH_4^+ \rightleftharpoons NH_3 + H^+ \qquad 3.44$$

with pK value of 9.2.

Ammonia N is a prime nutrient for algae and is rapidly converted into cell protoplasm. The hydrogen ions released by this biological process alters the equilibrium of carbonate species as follows:

$$HCO_3^- + H^+ \rightleftharpoons H_2CO_3 \rightleftharpoons CO_2 + HOH \qquad 3.45$$

The net reaction can be written as:

$$HCO_3^- + NH_4^+ \rightleftharpoons CO_2 + NH_3 + HOH \qquad 3.46$$

Algal uptake of CO_2 and ammonia would result in a net decrease in alkalinity. Continued removal of ammonia at a pH or near the pK value given by equation 3.44 may result in significant amount of hydrogen ions. Due to such release of H^+ ions, pH drops unless it is made up by other means. The decrease in pH and $CO_3^=$ ion concentration and the accompanying increase in HCO_3^- ion concentration suggest that the equilibrium has simply shifted according to the equation:

$$HCO_3^- \rightleftharpoons CO_3^= + H^+ \qquad 3.47$$

At pH 8.0-8.3, the free ammonia present is available for the growing organisms.

Abeliovitch et al. (1976) reported that significant penetration of ammonia (NH_3) starts only at pH 8.0 and increases with the elevation of pH. Natarajan (1970) quoted Shilo et al. having presented evidence that at high pH it is free ammonia (NH_3) and not NH_4^+ ions that is responsible for the lytic activity of diatoms. This is due to easy penetrability of unionised ammonia (NH_3) into the cells.

Loehr (1974) indicated that ammonia toxicity to aquatic organisms is increased markedly at low dissolved oxygen concentration. According to Hattori (1958) the injurious effect of ammonia and urea is due to the conversion of these nitrogen sources into soluble form inside the cell. Dohler (1971) reported that ammonia diffuses passively into the cells of

Chlamydomonas reinhardtii while nitrate is taken by active transport. Warren (1962) indicated that ammonia at low pH is usually toxic in overwhelming quantities whereas at high pH much smaller quantities may be lethal. It is from these observations enumerated that pH was fixed at 8.0-8.3 and aeration done in the course of this research.

3.7. Dairy Waste as an Organic Carbon Source:

Milk plant wastes are generally high in dissolved organic matter. Quirk et al. (1972) had reported that whey from the manufacture of soft or hard cheese contains a high concentration of 30000-50000 mg/l of BOD. They further reported that the BOD of whey is about 60 percent of COD. However, on dilution the whey effluent contains a total COD of 840-2340 mg/l at a pH of 5.5-7.0. Nemerow (1971) reported that BOD of whey is 32000 ppm whereas process wastes in general contain 1890 ppm of BOD. McKee (1950) documented that milk wastes have a BOD concentration as high as 2500 ppm.

According to Millen et al. (1975), the final effluent of milking centres contain 2100-2300 mg/l BOD and 4924-8000 mg/l total COD. The pH lies between 7.1-8.1. Total nitrogen content is reported to be 115-190 mg/l. Maloney et al. (1960) had given the composition of whey. Whey which averages 6.9 percent of total solids consists of 0.3 percent fat, 0.9 percent protein, 4.9 percent lactose, 0.6 percent ash and 0.2 percent lactic acid.

Lactose is readily oxidised by bacteria and has a short time, but high nitrogenous demand. Hoover et al. (1953) had shown that 1000 ppm of skim milk solids were completely converted to cells in 5-6 hours. The protein molecules are degraded only slowly. Maloney et al. (1960) further indicated that high oxygen demand and nitrogen deficiency affected the working of ordinary stabilization ponds. However, when large amount of nitrate was supplied, the ponds worked well.

Hoover et al. (1951) indicated that when sufficient nitrogen was supplied, lactose assimilation by activated sludge was rapid. Moreover the activated sludge biomass also increases in weight by 43 percent more than normal biomass. In the algal-bacterial system, which is proposed to treat high nitrogenous wastes under continuous aeration, milk wastes seem to afford a good source of organic carbon. Under well aerated conditions and in the presence of about 1000 mg/l of NH_3N, a small COD of the order of 4000 mg/l would be easily taken up by the biomass as a source of organic carbon instead of glucose.

3.8. Flocculating Algal-Bacterial System:

This is comparitively a new process, study of which have been conducted in Kansas University (U.S.A.) and in the Indian Institute of Technology, Kanpur (India). McGriff (1970) demonstrated the ability of algal-bacterial system to achieve excellent BOD, COD and nutrient removal rates in domestic

wastewater under light-dark cycle. Nitrogen removal is effected by ammonia stripping and metabolic assimilation and phosphorus is removed by biological precipitation and metabolic assimilation. To attain these results a mixed liquor suspended solids concentration of 1000-1400 mg/l is required.

John (1976) studied the aspects of domestic wastewater treatment by algal-bacterial system under natural sunlight. He concluded that flocculating algal-bacterial system is effective both under sunlight and light-dark cycles. The biomass flocculated well and settled readily. It is further reported the system is capable of removing 86 percent of COD, 89 percent of nitrogen and 90 percent of phosphorus with a biomass concentration of 1400-1600 mg/l. The solids retention time is reported to be 8-10 days. The predominant species are reported to be Chlorella. Under those conditions algae:bacteria ratio at a light intensity of 4300 lux was 60:40. It is indicated that this ratio did fluctuate with the fluctuation of light intensity.

4. THEORY AND RATIONALE OF THE PROPOSED PROCESS

4.1. Basic Assumptions Made:

Though the organic composition of bacteria (bacterial formula) had been reported to be varying under different environmental and nutrient conditions, the classical composition of bacteria as reported by Hoover et al. (1952) is taken for granted in this thesis. Degyansky et al. (1977) proposed the composition of $C_{60}H_{87}O_{23}N_{12}P$ for the bacterial culture in a nitrifying activated sludge system.

Hendricks et al. (1974) confirmed the formula $C_5H_7O_2N$ for bacteria. This has been the formula proposed by Hoover et al. (1952). McCarty, as quoted by Hendricks et al. (1974) also adopted the same formula for bacteria.

The same problem is encountered with regard to the formula for algae. Hendricks et al. (1974) adopted the formula as $C_8H_{14}O_3N$ for algae. Stumm et al. (1970) found out the formula for algae to be $C_{106}H_{263}O_{110}N_{16}P$. However Fogg (1952) in his classical book proposed the formula for algae as $C_{5.7}H_{9.8}O_{2.3}N$. The formula due to Fogg is considered in this thesis to represent algae.

Syrett (1972) found that the composition of algae is $C_{5.7}H_{9.8}O_{2.3}N$ irrespective of whether they are grown in nitrate of ammonia. Another important factor that was considered in the process development was the N:P ratio. There are various N:P ratios given for algae and bacteria depending upon environmental conditions.

Chiaudari et al. (1974) documented that in the growth of almost all algae neither phosphorus nor nitrogen was limiting when the N:P ratio was between 5 and 10. Floodgate (1973) reported that most phytoplankton have a C:N ratio of 6:1 to 7:1. Ryther et al. (1971) observed N:P ratio to be 30.9:1 in phosphorus deficient cells and 2.9:1 in nitrogen deficient cells of Chlorella pyrenoidosa.

Bogan et al. (1960) are of the opinion that assimilation of 1 mg/l phosphorus by algae would be accompanied by metabolism of 33-78 mg/l of carbon and 11-12 mg/l or more of nitrogen. Similar considerations also applied to bacteria. Sculze (1966) observed that for the removal of 100 mg/l BOD by biological

process, 6.8 mg/l nitrogen and 1.7 mg/l phosphorus are required for the building of the cell material.

Gibson (1971) found that the mean atomic ratio of N:P in algal cells ranges from 10:1 to 20:1 which corresponds to a weight ratio of 4.5:1 to 9:1. However Borchardt et al. (1968) reported that cell masses incorporate phosphorus much above critical level of 3 percent depending on pH and carbon stress. Kuhl (1967) reported that under phosphorus deficiency, the production of dry weight and total nitrogen is lower while carbohydrate content of the cells is significantly higher.

Kuhl (1972) quoted Scot having observed that Chlorella pyrenoidosa takes up phosphate and potassium in the ratio of 1 atom P to 1 atom K suggesting that both elements combine chemically with some cell constituent. He also found that in magnesium and manganese deficient cells there is an accumulation of phosphorus. Kuhl (1972) quoted Bodour having observed that potassium enhances phosphorus uptake in Chlorella vulgaris. Krauss et al. had been quoted by the above author that in a medium deficient of micro-nutrients the phosphorus content of the cells is appreciably higher than normal cells.

From the foregoing facts N:P ratio was kept at 10:1 throughout the experimental studies in the proposed system. However organic carbon dose was changed. When organic carbon is supplied, bacteria utilise the same quickly, while algae have to complete with bacteria to obtain their share. Some small

quantity of organic carbon is likely to be taken up by algae as well. Therefore an assumption is made that the organic carbon supplied is fully utilised by bacterial flora. In a mixotrophic algal-bacterial culture this might not be true. Still due to symbiotic attachment, even the organic carbon taken up by algae is likely to be recycled to bacteria.

4.2. Stoichiometry of the System:

According to Cramer et al. as quoted by Syrett (1972) the synthesis of algae from ammonium is as follow:

$$1.0\ NH_3 + 5.7\ CO_2 + 3.4\ H_2O \xrightarrow{\text{Light energy}} C_{5.7}H_{9.8}O_{2.3}N + 6.25\ O_2 \qquad 4.1$$

(Chlorella)

i.e. 17 parts of ammonia + 250.8 parts of carbon dioxide + water $\xrightarrow{\text{Light energy}}$ 129 parts of Chlorella + 100 parts of oxygen

or 129 mg of algae requires 17 mg of ammonia and 251 mg of carbon dioxide while releasing 100 mg of oxygen.

or 64 mg algae requires 8.5 mg of NH_3 + 126 mg CO_2

4.2

Coming to bacteria, the equation of bacterial synthesis proposed by Schroeder et al. as quoted by Hendricks et al. (1974) is adopted in this work. The equation is as follows:

$$6\ C_6H_{12}O_6 + 16\ O_2 + 4\ NH_3 \xrightarrow{\text{Energy}} 4\ C_5H_7O_2N + 16\ CO_2 + 28\ H_2O \qquad 4.3$$

(Bacteria)

i.e. 6 x 180 parts of glucose + 16 x 32 parts of oxygen + 4 x 17 parts of ammonia $\xrightarrow{\text{Energy}}$ 4 x 113 parts of bacteria + 16 x 44 parts of carbon dioxide + water

or 113 mg of bacteria requires 270 mg of glucose, 128 mg of oxygen and 17 mg of ammonia

Dividing the whole by 3, we get:

Approximately 37 mg of bacteria requires 90 mg of glucose, 43 mg of oxygen and 6 mg of ammonia 4.4

Now adding equations 4.2 and 4.4 we get:

64 mg of algae + 37 mg of bacteria — 100 mg of algal-bacterial mass

100 mg of algal-bacterial mass requires the following:

	8.5 mg ammonia
gives out 50 mg O_2	126 mg carbon dioxide
	90 mg glucose
	6 mg ammonia
gives out 59 mg CO_2	43 mg oxygen.

Total requirement for 100 mg of algal-bacterial biomass = 14.5 mg ammonia or 12 mg N; 90 mg glucose or 36 mg C; 67 mg carbon dioxide.

The N:P ratio as discussed earlier is taken as 10:1. Hence the amount of phosphorus required is:

$$\frac{1}{10} \text{ Nitrogen}$$

$$= \frac{14.5}{1.22 \times 10} = 1.2 \text{ mg P}$$

Organic carbon in 90 mg of glucose is 36 mg of carbon. Hence C:N:P ratio of the feed is to be of the order of 36:12:1.2

or 30:10:1.

Hence the organic carbon and phosphorus has to be added depending on the quantity of nitrogen in the feed to make it a balanced one. Besides carbon dioxide is to be supplemented. This is done by aeration.

Normally the quantity of free CO_2 present in water is 0.4-1.0 mg/l (Kuentzel, 1969; Manahan, 1974). Taking 0.4 mg/l as the quantity that will be available by bubbling air, the quantity of air required can be calculated. Here the alkalinity has also to be taken into account.

At a pH below 8.3 only bicarbonate alkalinity will be available. So, out of total 480 mg/l of alkalinity in the tap water used, only 240 mg/l (half of the total bicarbonate alkalinity) will be available for algae as per equation:

$$2\ HCO_3^- \rightleftharpoons CO_3^= + H_2O + CO_2 \qquad 4.5$$

Totally 67 mg of CO_2 is required per 100 mg of biomass as per calculations shown earlier. Assuming a detention time of 2 days, the quantity of aeration can be calculated as follows:

Alkalinity in 100 ml of water = 48 mg

Alkalinity available for algae = $\frac{1}{2}$ x 48 mg

= 24 mg as $CaCO_3$

Therefore alkalinity available as CO_2 = 106 mg

The balance of carbon dioxide required = 67 - 106 = 56 mg

Aeration is done for 48 hours to obtain 56 mg of CO_2.

Total quantity of air required to supply 47 mg of CO_2 = $\frac{56}{0.4}$ = 140 l

Aeration per minute per 100 mg of biomass $= \frac{1}{24 \times 60} = \frac{1}{24}$ lpm.

Hence for the culture of 100 mg of algal-bacterial biomass, the following are required:

14.5 mg of ammonia or 12 mg of N

90 mg of glucose or 36 mg of C

1.2 mg of P

$\frac{1}{24}$ lpm rate of aeration to provide CO_2.

4.3. Biomass Requirement for Treatment:

Normally the total ammonia and urea in the fertiliser wastes have been found to be of the order of 700 mg/l NH_3 and 500 mg/l urea. This works out to be roughly about 1000 mg/l N hence to treat 1000 mg/l N, the biomass required

$$= \frac{100 \times 1000}{12.0} = 8300 \text{ mg/l}$$

since 100 mg of biomass requires 12.0 mg of ammonia N.

If the feed contains more of ammonia N, then the biomass concentration has to be increased. Actually in the experiments, upto 1200 mg/l NH_3 (1000 mg/l N) was tried. Moreover, this much biomass may build up if 100 percent removal of nutrients takes place. This, however, does not happen. So the biomass developed will depend upon the efficiency of nutrient removal.

For 1000 mg/l of biomass the following nutrients are required

Ammonia	=	1000 mg/l NH_3N
Glucose	=	6.3 g/l
Phosphorus	=	84 mg/l P
Aeration to provide CO_2	=	$\frac{7000}{100 \times 24}$ = 3.0 lpm

During night, respiration will be there and there is a build up of CO_2 in the alkalinity system and in fact during night aeration CO_2 gets saturated and only during day time CO_2 is required for photosynthesis. Moreover, during night at low temperature, the CO_2 concentration is more in the air and all these quantities replenish the destroyed bicarbonate alkalinity. Hence only about two thirds of the calculated rate was provided arbitrarily. So aeration at a rate of 1 lpm was done and was found to support the growth well.

When urea is also fed, the breakdown action by bacteria would be as follows:

$$CO(NH_2)_2 + H_2O \rightarrow CO_2 + 2NH_3$$

or 60 parts of urea → 44 parts of CO_2 + 34 parts of NH_3

or 34 mg of NH_3 → 60 mg urea

or 340 mg of NH_3 is released by 600 mg of urea.

In a few sets of experiments urea and ammonia were used as nitrogen sources. Instead of 1000 mg/l NH_3N, 700 mg/l NH_3N and 300 mg/l NH_3N equivalent were used.

$$300 \text{ mg/l } NH_3N \equiv \frac{34}{60} \times A \text{ mg/l urea}$$

where A = Urea concentration (mg/l)

Therefore $A = \frac{300 \times 60}{34} = 530$ mg/l

So approximately 700 mg/l NH_3N and 500 mg/l urea were added to make up the total equivalent to 1000 mg/l NH_3N.

4.4. Batch Reactors in Series:

The effect of retention time on the concentration of pollutants leaving well mixed reactors or lagoons can be calculated from their rates of degradation. Assuming the rates of degradation following a first order reaction:

$$\frac{dC}{dt} = -kC \qquad 4.6$$

where, C = Concentration of ammonia

k = Its degradation or use by algae

t = Time.

Let there be n number of reactors each with a detention time T. Effective removals could be obtained by:

(1) Increasing the detention period

(2) Increasing the number of reactors.

The rate of change of concentration in the effluent from a well mixed reactor can be represented as:

Observed rate of change = Rate of entry − Rate of loss due to degradation − Rate of loss due to flushing

$$\left(\frac{dC}{dt}\right)_o = \left(\frac{dC}{dt}\right)_e - \left(\frac{dC}{dt}\right)_d - \left(\frac{dC}{dt}\right)_f \qquad 4.7$$

where, $\left(\frac{dC}{dt}\right)_e = \frac{C_o}{T}$ 4.8

$$\left(\frac{dC}{dt}\right)_d = -kC \qquad 4.9$$

and $\left(\frac{dC}{dt}\right)_f = \frac{C}{T}$ 4.10

where, C_o = Concentration of ammonia in the influent

C = Concentration of ammonia in the effluent.

Under steady state conditions $\frac{dC}{dt} = 0$.

or $\frac{dC}{dt} = 0 = \frac{C_o}{T} - kC - \frac{C}{T}$ 4.11

or $C\left(k + \frac{1}{T}\right) = \frac{C_o}{T}$ 4.12

or $\frac{C}{C_o} = \frac{1}{T\left(k + \frac{1}{T}\right)} = \frac{1}{(kT + 1)}$ 4.13

This is the case for first reactor. In the second reactor the concentration of influent is $\frac{C_o}{(kT + 1)}$ and applying the same logic as above the concentration of ammonia in the effluent of second reactor is calculated as:

$$\frac{C_2}{C_o} = \frac{1}{(kT + 1)(kT_2 + 1)} \qquad 4.14$$

For n reactors

$$\frac{C_n}{C_o} = \frac{1}{(kT + 1)(kT_2 + 1)(\ldots)(kT_n + 1)} \qquad 4.15$$

So, instead of one single reactor of large size, if it is divided into n number of reactors of equal volumes with identical detention times of $\frac{T_n}{n}$, then

$$\frac{C_n}{C_o} = \frac{1}{\left(\frac{kT_n}{n} + 1\right)^n} \qquad 4.16$$

Therefore

$$C_n = \frac{C_o}{\left(\frac{kT_n}{n} + 1\right)^n} \qquad 4.17$$

4.4.1. Evaluation of k.

Assuming that the reaction is a first order one, a rectangular coordinate plot of the observed effluent concentration $\overline{C}$ versus the calculated rate of reaction under steady state conditions yields a linear trace with a slope k and an intercept of zero.

The equation

$$\frac{\overline{C}}{C_o} = 1 + \frac{k(\overline{C})V}{QC_o} \qquad 4.18$$

where $\overline{C}$ = the steady state value of effluent concentration, C, $k(\overline{C})$ = rate of reaction.

In a first order reaction:

$$k(\overline{C}) = - kC \qquad 4.19$$

For certain volume of the reactor and various flow rates and influent concentrations, the steady state effluent concentrations are found out. Then the value of $-k(\overline{C})$ is calculated from the equation 4.19. The values of $\overline{C}$ and $-k(\overline{C})$ are plotted and a linear plot is obtained. The slope of this line gives the value of k.

4.5. Continuous Flow Reactor:

The continuous flow reactor is worked so as to have a biomass concentration of about 7000 mg/l at optimum C:N:P ratios of 30:10:1. However, at low C:N:P ratios, this biomass concentration is likely to drop to the level of 4000 mg/l or so.

4.5.1. Evaluation of Biokinetic Constants:

The net change of the substrate in a reactor is expressed as:

$$\text{Net change} = \text{Inflow} - \text{Outflow} - \text{Consumption}$$

or

$$V\left(\frac{dS}{dt}\right)_{net} = FS_o - FS_1 - V\left(\frac{dS}{dt}\right)_{growth} \qquad 4.20$$

But we know that

$$\frac{dX}{dt} = \mu X \quad \text{(Sherrad et al, 1975)} \qquad 4.21$$

and $$\frac{dX}{dt} = Y(-\frac{dS}{dt}) \qquad 4.22$$

Combining equations 4.20 and 4.22, we get:

$$(-\frac{dS}{dt}) = \frac{dX}{dt} \cdot \frac{1}{Y} = \frac{\mu X}{Y} \qquad 4.23$$

Substituting this in equation 4.20 at steady state conditions when net growth is zero i.e.

$$V(-\frac{dS}{dt}) = 0 \qquad 4.24$$

Therefore $$V \frac{\mu X}{Y} = F(S_o - S_1) \qquad 4.25$$

or $$\mu X = \frac{Y(S_o - S_1)}{\theta} \quad \text{as} \quad \theta = \frac{V}{F} \qquad 4.26$$

where, X = Concentration of organisms (mg/l)

t = Time

$\bar{\mu}$ = Maximum specific growth rate

μ = Specific growth rate

S = Substrate concentration (mg/l)

K_s = Saturation constant defined as substrate concentration at which $\mu = \frac{\bar{\mu}}{2}$

Y = Yield coefficient

k_d = Microorganism decay rate

F = Flow rate.

We know that:

$$\mu = \frac{1}{\theta} + kd = \frac{1 + k_d\theta}{\theta} \qquad 4.27$$

Then

$$X = \frac{Y(S_o - S_1)}{\theta \mu} = \frac{Y(S_o - S_1)}{(1 + k_d\theta)} \qquad 4.28$$

The effluent substrate concentration can be obtained from:

$$\mu = \frac{1}{\theta} + k_d = \bar{\mu} \frac{S_1}{K_s + S_1} \qquad 4.29$$

inverting this equation, namely, 4.29, we get:

$$\frac{\theta}{1 + k_d\theta} = \frac{K_s}{\bar{\mu} S_1} + \frac{S_1}{\bar{\mu} S_1} \qquad 4.30$$

Solving for S_1, we get:

$$S_1 = \frac{K_s(1 + k_d\theta)}{\theta\bar{\mu} - (1 + k_d\theta)} \qquad 4.31$$

The equations for predicting the performance of a completely mixed reactor can be seen in equations 4.28 and 4.31. Inverting and rearranging the equations, we get:

$$\frac{S_o - S_1}{X_1} = \frac{k_d\theta}{Y} + \frac{1}{Y} \qquad 4.32$$

and

$$\frac{\theta}{1 + k_d\theta} = \frac{K_s}{\bar{\mu} S_1} + \frac{1}{\bar{\mu}} \qquad 4.33$$

Both these equations are of the form $y = mx + b$. Equation 4.32 should be produced as straight line with an intercept of $\frac{1}{Y}$ and a

slope of $\frac{k_d}{Y}$. The line is obtained when $\frac{S_o - S_1}{X_1}$ is plotted against θ. Once k_d has been evaluated from the above plot $\frac{\theta}{(1 + k_d\theta)}$ can be plotted against $\frac{1}{S_1}$ to produce a straight line for equation 4.33. The intercept of this line is $\frac{1}{\mu}$ and the slope is $\frac{K_s}{\mu}$. Thus the biokinetic constants are found out.

5. EXPERIMENTAL METHODOLOGY

5.1. Aim of the Present Research:

The aim of the present research work was to study the various parameters and system efficiency by using flocculating algal-bacterial system for the treatment of high nitrogenous wastes as it was already found that the system is inexpensive and efficient in the treatment of domestic wastes, especially in developing countries (John, 1976). The study was divided into three phases.

Phase 1 essentially consisted of batch studies. Parameters such as biomass concentration, algae/bacteria ratio, detention time, total N removal efficiency, COD removal efficiency, settling values and effluent biomass concentration were involved in the studies. Primarily glucose was used as a source of organic carbon. But it was replaced by an industrial waste (whey) from a cheese manufacturing concern. The highly encouraging results obtained therefrom are given in Chapter 7.

Effects of sewage as organic carbon source and that of 1 percent carbon dioxide on the total N removal efficiencies were studied. Tapwater, which was used throughout the experimental studies would provide essential micronutrients for the growth of biomass. Effect of pH on total N removal efficiency was also taken up.

C:N:P ratios ranging from 30:10:1 to 3:10:1 were studied under various conditions. C:N:P ratio of 30:10:1 is the stoichiometric ratio as derived in Section 4.2. As this high ratio would involve too much of organic carbon, lesser quantities were tried.

Phase 2 study was projected to increase the total N removal efficiency by providing 3 batch reactors working in series. These were run with a feed having a C:N:P ratio of 10:10:1 and total N concentration 1000 mg/l. This study was undertaken as the efficiency of single batch reactor was not sufficient enough to produce an effluent of acceptable standard.

Phase 3 study was performed on the working of a single continuous flow reactor with a feed containing 1000 mg/l of N (Ammonia N 700 mg/l + Urea N 300 mg/l). The C:N:P ratio of the feed was 10:10:1. The reactor was run with a liquid detention time of 1 day and SRTs of 10, 8, 6 and 4 days. The reactor was run under each SRT for a fortnight or more so that steady state conditions were attained. Biokinetic constants of the system were then determined.

5.2. Development of Stock Cultures:

Separate cultures of algae and activated sludge were developed independently and maintained throughout the experimental study.

5.2.1. Activated Sludge:

Two,3 litre beakers were used to develop activated sludge. A handful of fertile garden soil was taken and was put in a 1 litre beaker. The contents were mixed well with water and allowed to settle. The supernatant was poured into the 3 litre beakers. The soil was washed two or three times with a little quantity of water and poured into the culture vessels after allowing the contents to settle. Sewage from the sewage pumping station near the Air-strip of Indian Institute of Technology, Kanpur was brought daily and added to the culture vessel. The mixture of garden soil extract and sewage was aerated by means of cintered glass diffusers supplied by NEERI (India). The average characteristics of sewage used was as given in Table 9.

Table 9 - Average Characteristics of Sewage

Characteristics	Concentration, mg/l
pH	7.8
Total solids	850
COD	190
Total nitrogen as N	8.0
Total phosphorus as P	4.0
Total alkalinity as $CaCO_3$	480

Aeration was continued and every morning the biomass was allowed to settle for one hour. The supernatant was syphoned off and fresh sewage fed. 1.0 g each of glucose and a pinch of peptone were added in the initial stages to supplement organic carbon and nitrogen. Good flocs were formed after about a month. The biomass concentration reached a value about 6000 mg/l. Afterwards a part of the sludge was wasted so as to keep the reactor run well with a biomass concentration in the range of 4000-6000 mg/l. Daily feeding and wasting was continued. These culture vessels served as sources of activated sludge.

5.2.2. Stock Culture of Algae:

Algal culture was developed from the seed obtained from the Central Rice Research Institute, Cuttack (Orissa). Two long glass cylinders 15 cm diametre and 150 cm long kept at the courtyard behind the Environmental Engineering Laboratory of I.I.T. Kanpur served as culture vessels.

A loopful of the culture was taken and inoculated into a 3 litre beaker containing essential nutrients in sewage. The nutrients added were: 100 mg/l of urea, 20 mg/l of potassium phosphate, 5 mg/l of magnesium sulphate, 5 mg/l of ferric chloride and 100 mg/l of soda ash. The culture vessel was kept outside and aerated continuously. The colour of the contents became greenish in about 5 days. The contents were then transferred to the columns fed with sewage. The contents of the column were not aerated. There was profuse growth of algae in the columns

in a period of 7 days. The biomass concentration was of the order of 200-300 mg/l. Certain quantity of the mixed liquor was thrown out daily through ports on the side of the column. Equal volume of sewage was added.

5.2.3. Flocculating Algal-Bacterial Culture:

Before starting the feasibility studies, stock culture of flocculating algal-bacterial biomass was prepared. Settled activated sludge was taken and mixed with tapwater and its concentration determined. The characteristics of tapwater are given in Table 10.

Table 10 - Average Characteristics of Tapwater at I.I.T. Kanpur

Characteristics	Concentration, mg/l
pH	7.9-8.1
Total alkalinity as $CaCO_3$	460-510
Total calcium as $CaCO_3$	130

The concentration of activated sludge was brought to 2500 mg/l. The algal culture from the glass column was drawnoout and its concentration determined. It was 280 mg/l. One litre of the algal culture was centrifuged and the residue resuspended in 1.5 litres of original algal culture to obtain a biomass concentration of 460 mg/l. The previously made activated sludge

and the algal culture were mixed together. Then the contents were aerated. This mixture contained about 1000 mg/l of activated sludge and 280 mg/l of algae. This ratio has been fixed as on the lines of the work done by Humenik et.al. (1971) and John (1976). Aeration was continued. Biomass settling was observed everyday. Initially activated sludge alone settled while algae were in suspension. So 500 ml of mixed liquor was wasted daily while equal amount of sewage was fed. 1 litre of mixed liquor was wasted after a week and equal amount of sewage added. Algal-bacterial flocs became apparent at the end of 2 weeks. The wasting and adding of sewage continued for about 6 weeks, when good algal-bacterial flocs were found to take shape. Settling was quite good. Supernatant was more or less clear. The biomass concentration was about 1200 mg/l.

From this seed culture, mass culture was developed in 4 reactors by feeding sewage daily and syphoning out the supernatant. A biomass concentration of 1400-1500 mg/l was obtained in each reactor at the end of 10 days. The contents were allowed to settle and the supernatant syphoned out.

The flocs in all 4 reactors were mixed together and equal volumes of sludge added to the reactors. Feed was added to these and the volume made upto 2.5 litres and feasibility studies started.

5.3. Phase 1 - Batch Reactor Studies:

This consisted of 7 parts, which are described in detail in the following sections.

5.3.1. Phase 1 - Part 1: Feasibility Study:

3 litre beakers were used as reactors. The set up is shown in Figure 10. Ammonia N was added gradually in Reactor 1 just to sustain the biomass growth. 100 mg/l of ammonia N along with glucose and phosphate was fed in Reactor 1 so as to conform with C:N:P ratio of 30:10:1. The ammonia N dose was calculated such that 6 percent of N was provided in the above case. 300 mg/l, 400 mg/l and 500 mg/l of ammonia N along with required quantity of glucose and phosphate were added in Reactors 2, 3 and 4 respectively. Aeration was done at 1 lpm/l of reactor contents. The reactors were kept in the open yard. Light was falling on the reactors for about 7 hours during summer and for 10 hours during winter due to the shading of the Environmental Engineering Laboratory. The visible solar radiation ranged from 89 - 292 Langleys per day (John, 1976). Aeraration was stopped after 2 days and the contents allowed to settle for 1 hour. The supernatant was syphoned off. Fresh feed containing ammonia, glucose and phosphate in tapwater were added to all reactors and the volume made up to 2.5 litres. Aeration was continued. The same procedure was repeated every days and feed concentrations increased, maintaining the same C:N:P ratio. Feeds containing ammonia N concentration of 1000 mg/l were added to Reactors 2, 3

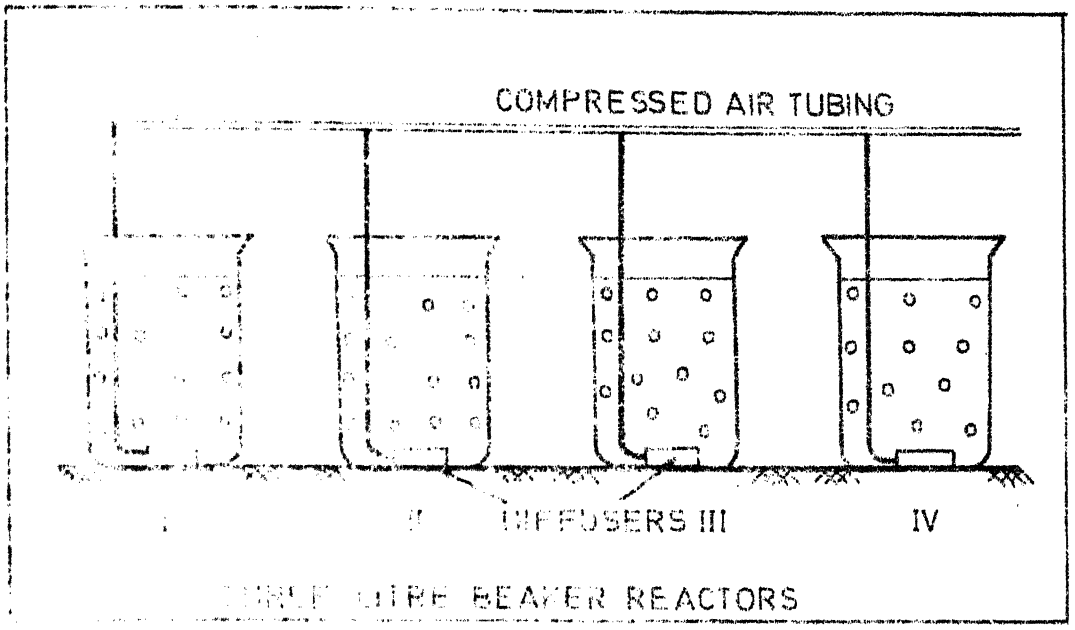


FIG. SET UP IN PHASE I STUDIES

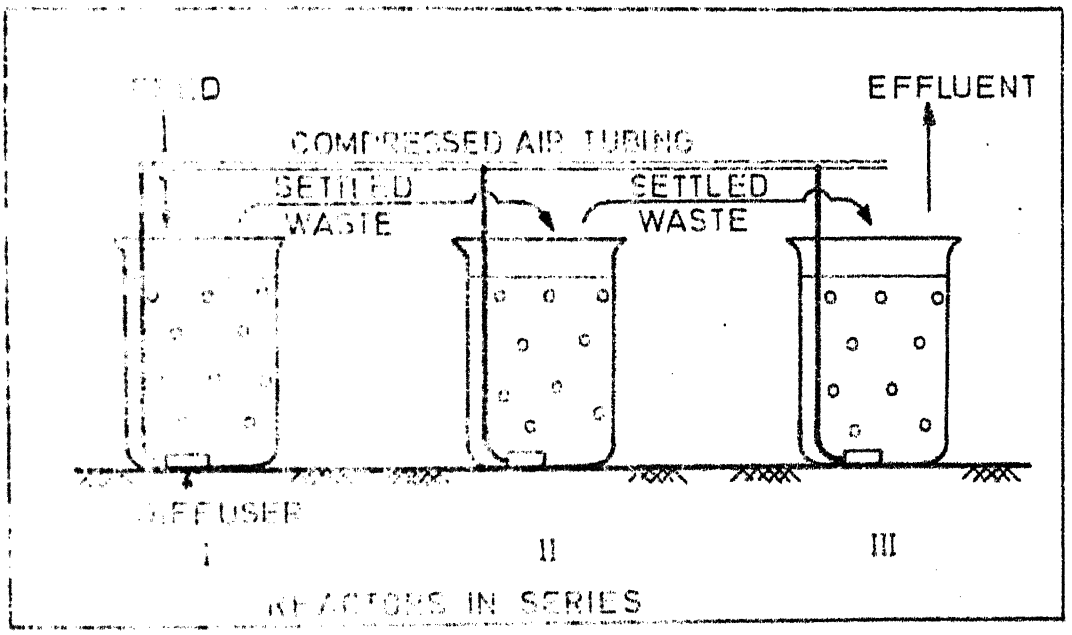


FIG.II SET UP IN PHASE II STUDIES

and 4 at the end of 12 days. The feed for Reactor 1 contained 250 mg/l of ammonia N. The reactors were then aerated for 6 days without any more feed.

Analyses for COD, ammonia N, nitrite N, nitrate N, pH and alkalinity were carried out. Effluent biomass concentration, biomass concentration and algal concentration were also determined. Values obtained after allowing the contents to settle in 1 litre measuring cylinder for half an hour were also determined. Afterwards Phase 1 - Part 2 study was taken up.

5.3.2. Phase 1 - Part 2: Ammonia Removal Study with C:N:P Ratios of 30:10:1, 25:10:1, 20:10:1 and 15:10:1:

Four reactors were set up in this study. The C:N:P ratios of feed in Reactors 1, 2, 3 and 4 were 30:10:1, 25:10:1, 20:10:1 and 15:10:1 respectively. This study was carried out to know how reduced quantity of organic carbon would affect the total N removal efficiently and the biomass stability. From the point of view of economy reduced quantity of organic carbon would be welcome and hence the attempt.

The acclimatised culture from Phase 1 - Part 1 study was washed well and allowed to settle. The supernatant was syphoned off and the sludge in Reactors 2, 3 and 4 were mixed and equal volumes of the same poured in all the 4 reactors. Glucose, ammonia and phosphate were taken in required quantities so as to conform with the C:N:P ratios and mixed in tapwater. The biomass concentration was about 5000-6000 mg/l to start with.

Reactor contents were then aerated for 2 days after feeding. Then aeration was stopped, contents allowed to settle for 1 hour and the supernatant syphoned off completely. The same feed as initial one was given and volume made up to 2.5 litre mark in each reactor. Aeration was continued for 2 days. This procedure was repeated for 12 days. A little of biomass was wasted each time such that the biomass left behind was about 5000-6000 mg/l.

As in Chapter 4 the biomass required for the removal of 1000 mg/l ammonia N is about 7000 mg/l. The present biomass concentration was fixed as such to have uniformity as the biomass concentration never went beyond 5000-6000 mg/l in reactors having lower C:N:P ratios. Then the feed was again introduced and the reactors run for 10 days continuously without any further addition of feed. However everyday tapwater was added to make up the evaporation losses. Analyses to determine COD, ammonia N, nitrite N, nitrate N, alkalinity, pH, biomass concentration, algae concentration, settling value and effluent biomass concentration were carried out at the end of every 2 days.

5.3.3. Phase 1 - Part 3: Ammonia Removal Study with C:N:P Ratios of 20:10:1 and 15:10:1 with and without Carbon Dioxide:

Having found out that a C:N:P ratio of 20:10:1 would be sufficient to support a biomass concentration of about 6000-7000 mg/l from Phase 1 - Part 2 study, it was decided to see whether 1 percent carbon dioxide in air has any effect on the total N

removal efficiency. 4 reactors were set up. Feeds with C:N:P ratios of 20:10:1 and 15:10:1 with and without carbon dioxide were given to various reactors. Acclimatisation was done for about 12 days till a steady state condition was achieved as in earlier experiments. Excess biomass was wasted every 2 days. Then feeding was done, volume made up to 2.5 litres and bubbling of air and carbon dioxide began. Readings at the end of every 2 days were taken up to 10 days. The experiment was then terminated.

5.3.4. Phase 1 - Part 4: Ammonia Removal Study with C:N:P Ratios of 14:10:1, 6:10:1, 3:10:1 and Sewage:

This study was carried out to explore the possibilities of reducing the organic carbon to the least extent without affecting total N removal efficiency and biomass stability. From Phase 1 - Part 3 it was found that carbon dioxide bubbling did not significantly enhance the total N removal efficiency. So carbon dioxide was not used in later experiments.

C:N:P ratios of 14:10:1, 6:10:1 and 3:10:1 were tried in this experiment. Domestic sewage with organic carbon supplement was fed in Reactor 4. The C:N:P ratio was approximately 3:10:1. The domestic waste was having a COD of the order of 190 mg/l and therefore the study was not attempted for higher C:N:P ratios. A biomass concentration of 6000 mg/l was fed to all reactors. Then feed was given and acclimatisation was carried out as in earlier experiments. However at the end of acclimatisation the

biomass in reactors except that in Reactor 1 never rose beyond 4000 mg/l. So no sludge was wasted from Reactors 2, 3 and 4. After applying fresh feed the experiment was started. Analyses for various parameters were carried out as in earlier experiments. The experiment was terminated after 10 days and Phase 1 - Part 5 study started.

5.3.5. Phase 1 - Part 5: Ammonia Removal Study with C:N:P Ratio of 6:10:1 with pH at 8.0-8.3:

It was observed from Phase 1 - Part 4 study that a total N removal efficiency of 38 percent could be achieved at a C:N:P ratio of 6:10:1 and with a biomass concentration of 4400 mg/l. The temperature range was 12-19°C during the experiment. The total N removal efficiency was too low at a C:N:P ratio of 3:10:1; and neither was it too high at a C:N:P ratio of 14:10:1. So the effect of pH on total N removal efficiency at a C:N:P ratio of 6:10:1 was studied in this experiment. Reactor 1 was kept as a control with no pH adjustment. A pH of 8.0-8.3 was maintained in Reactors 2 and 3 by adding $Ca(OH)_2$ and Na_2CO_3 respectively. Acclimatisation and feeding were accomplished as in earlier experiments. Initial biomass concentration was 4700 mg/l. Analyses were carried out as done in earlier experiments.

5.3.6. Phase 1 - Part 6: Ammonia Removal Study with Activated Sludge System, Algal System and Flocculating Algal-Bacterial System and Simple Aeration:

To know how for flocculating algal-bacterial system is effective in total N removal, in comparison with activated sludge system,

algal system and simple aeration, this study was undertaken. Experiment was carried out with a feed C:N:P ratio of 6:10:1 and initial total N concentration of 1000 mg/l. No pH control was attempted. Almost pure algal culture was obtained by continuously aerating the algal biomass with 5 percent CO_2 in air, for a couple of weeks. Acclimatisation of activated sludge also was done earlier. 2.5 litre bottles were used as reactors. The experiment was started and again the biomass in various reactors acclimatised to the feed. Initial biomass concentrations were 5000 mg/l in Reactor 1, 3500 mg/l in Reactor 2 and 4200 mg/l in Reactor 3 respectively. Feeds were introduced and analyses carried out every 2 days as done in earlier experiments. Thereafter the experiment was terminated.

5.3.7. Phase 1 - Part 7: Urea and Ammonia Removal Study with C:N:P Ratios of 5:10:1, 7:10:1, 9:10:1 and 10:10:1:

Ammonia was the sole source of nitrogen in all the previous experiments in Phase 1. However urea along with ammonia was introduced in this study. The feed contained about 700 mg/l of ammonia N and 300 mg/l of urea N. The C:N:P ratios tried were 5:10:1, 7:10:1, 9:10:1 and 10:10:1 as urea degradation is accomplished by bacterial flora. Algae can effectively remove only ammonia from a feed containing ammonia and urea (Hattori, 1957; McCarthy, 1971 and Williams et.al., 1977). So higher C:N:P ratios were adopted in Reactors 2, 3 and 4 as to provide favourable conditions to the bacterial flora which would degrade urea. Acclimatisation was done as in earlier experiments.

The biomass concentrations after acclimatisation were between 4000-4500 mg/l. Experiment was started after feeding and aerating the reactor for 10 days. Analyses were carried out as done in earlier experiments. Sludge COD, sludge N and effluent organic N were determined in addition. This was done to find out the nitrogen mass balance. With this experiment Phase 1 studies were terminated.

5.4. Phase 2: Ammonia and Urea Removal Study with Batch Reactors in Series:

This study was conducted with a view to produce an effluent of high quality from high nitrogenous wastes. Three batch reactors were set in series as single batch reactors were found to be incapable of producing effluent of acceptable quality. The set up is shown in Figure 11. The feed contained 700 mg/l of ammonia N and 300 mg/l of urea N at a C:N:P ratio of 10:10:1.

Feeding was given to Reactor 1 and aeration continued for 2 days. Then the supernatant of Reactor 1 obtained after settling for 1 hour was transferred to Reactor 2. Organic carbon supplement was provided so as to bring the C:N:P ratio to 10:10:1. Details of addition are given in Appendices E.1, E.2 and E.3. Feeding was given to Reactor 1 and both the reactors were aerated for 2 days. Aeration was stopped and the contents allowed to settle. The supernatant of Reactor 2 was transferred to Reactor 3 and that of Reactor 1 to Reactor 2. Organic carbon was supplemented to the influents of Reactors 2 and 3. This was repeated for

7 or 8 cycles. Sludge wasting was done every 2 days so as to bring the biomass concentration in the range of 5000-6000 mg/l.

5.4.1. Phase 2 - Part 1: Ammonia and Urea Removal Study with Batch Reactors in Series - Biomass Concentration 5000 mg/l and no pH Adjustment:

The reactors were run for another 10 days as described in the previous section. The biomass concentration in all the three reactors was maintained in the range of 5000-6000 mg/l. Analyses were done at the end of every 2 days as described earlier. The experiment was then terminated.

5.4.2. Phase 2 - Part 2: Ammonia and Urea Removal Study with Batch Reactors in Series - Reduced Biomass Concentrations in Reactors 2 and 3 and no pH Adjustment:

As high biomass concentrations in Reactors 2 and 3 seemed to have adverse effect on total N removal efficiency, biomass concentrations of 1500-1600 mg/l were tried in Reactors 2 and 3. However, the biomass concentration in Reactor 1 was maintained around 5000 mg/l as in the earlier experiment. The procedures of feeding and acclimatisation were exactly same as those in Phase 2 - Part 1. Experiment was run for 10 days. Analyses were performed at the end of every 2 days. Then the experiment was terminated.

5.4.3. Phase 2 - Part 3: Ammonia and Urea Removal Study with Batch Reactors in Series - Reduced Biomass Concentrations in Reactors 2 and 3 with pH at 8.0-8.3:

Reactors in this experiment was run as that in Phase 2 - Part 2. But pH was adjusted everyday by adding the required quantity of

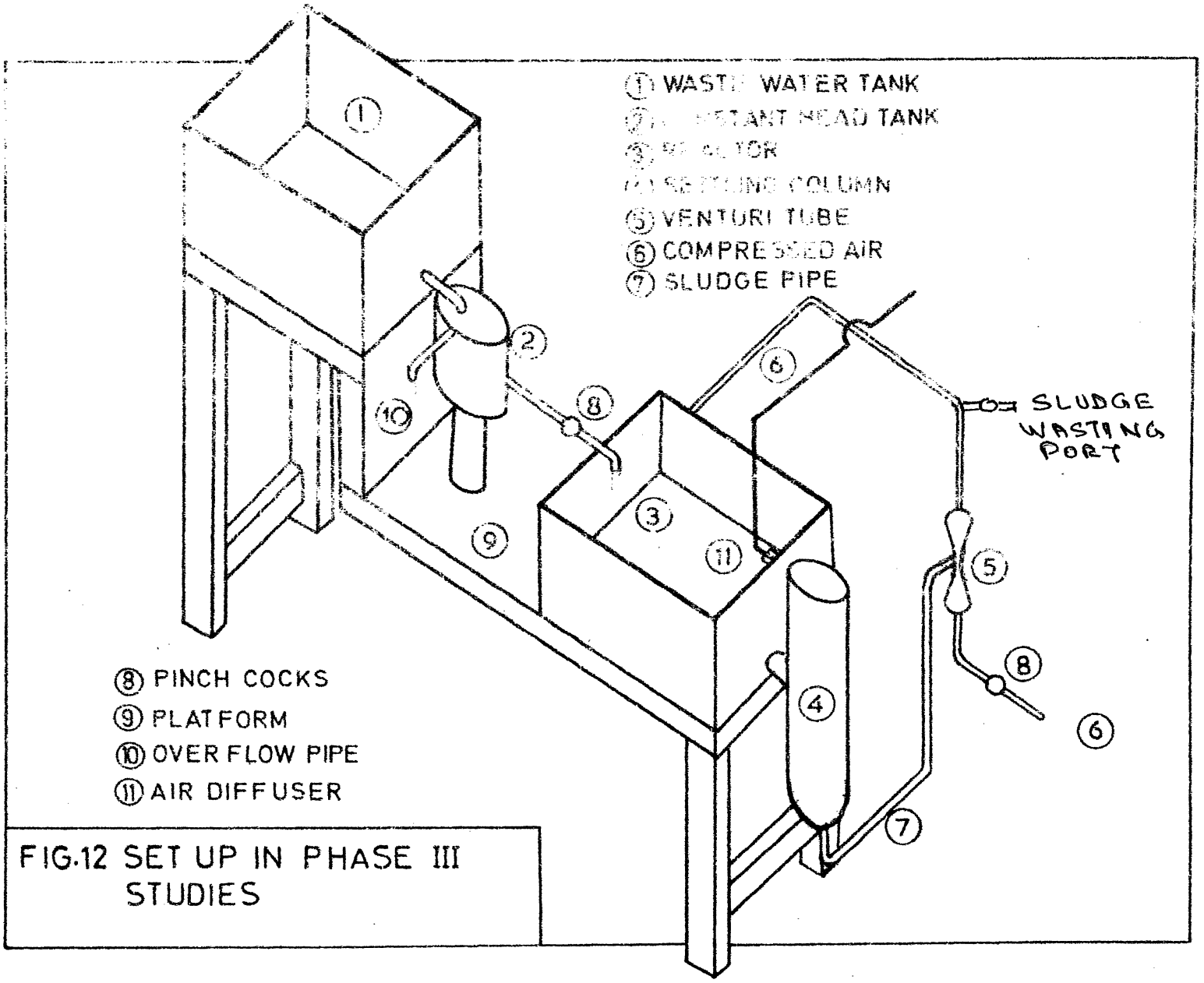
Na_2CO_3 to all the reactors so as to bring the pH in the range of 8.0-8.3. Analyses were performed every 2 days.

5.4.4. Evaluation of k:

The value of k was determined in the batch reactors after proper acclimatisation. One reactor was operated with no pH control while the other was with pH control. The feed was given and the effluent total N concentration was determined every 2 days. Then the values were determined at 3 days' detention time. The effluent total N concentrations were determined at detention times of 4 and 6 days. The values were tabulated as shown in Section 6.6.4. The value of k was then determined by drawing a graph with $k(\overline{C})$ along y axis and $\overline{C}$ along x axis, where $\overline{C}$ was the effluent total N concentration at steady state condition and $k(\overline{C})$, the rate of reaction. From the value of $k(\overline{C}) = k\overline{C}$ as per equation 4.19 we get $k = k(\overline{C})/\overline{C}$. The slope of the line gives the value of k. Values of k were determined for Reactor 1 with no pH control and for Reactor 2 with pH at 8.0-8.3.

5.5. Phase 3 - Continuous Flow Single Reactor Study:

This experiment was carried out to evolve the biokinetic constants. The set up used for this experiment is as shown in Figure 12. The reactor was a rectangular perspex box of dimensions 20 cm x 20 cm x 17.5 cm. 8.5 cm diameter and 75 cm deep glass settling column was attached to the reactor. Sludge recirculation was done by means of compressed air through a



12

FIG.12 SET UP IN PHASE III STUDIES

venturi tube. Sludge wasting was done only once every morning as it was not possible to waste the sludge continuously. The reactor was run for a month and then the experiment started. 10 percent of sludge was wasted to have a SRT of 10 days. This was done daily. Continuous feeding was done by means of a feed tank and a constant head tank. The liquid detention time was 1 day. Flow rate was adjusted accordingly, every 5 to 6 hours. After running the reactor for 15 days till steady state condition was achieved, the influent and effluent characteristics were determined. Then the SRT was changed to 8 days with sludge wasting of 12.5 percent. The reactor was run for 15 days to come to a steady state condition. Analyses were carried out as done in the earlier case. After this experimental observations for SRTs of 6 and 4 days were taken. The experiment was then terminated.

With the known values of SRTs and other parameters, the bio-kinetic constants such as Y, k_d, $\bar{\mu}$ and K_s were determined, where

Y = Yield coefficient

k_d = Microorganism decay rate

$\bar{\mu}$ = Maximum specific growth rate, and

K_s = Saturation constant at which $\mu = \frac{\bar{\mu}}{2}$.

5.6. Analytical Methods:

Brief descriptions of the analytical methods used are given herein. However, the techniques as described in Standard Methods

(1965) are not described. The methods adopted are only indicated in such cases.

5.6.1. Instruments Used:

The instruments used in various analyses are given below:

(a) Centrifuge (Sorvall SS 3 auto.)	:	Ivan Sorvall Inc. Norwalk, Connecticut, USA
(b) Kjeldar Apparatus	:	Narang Scientific Works New Delhi
(c) Microscope	:	Bausch and Lomb Inc. Rochester, New York, USA
(d) Millipore Filter Assembly and Filter Paper	:	Millipore Corporation Bedford, Messachussetts, USA
(e) pH Meter	:	Philips India Ltd. Bombay
(f) Spectrophotometer (Spectronic-20)	:	Bausch and Lomb Inc. Rochester, New York, USA

5.6.2. Sampling and Analyses:

Samples were collected from the reactors once in 2 days at about 9.00 AM. Analyses were carried out immediately after collection. Various analytical procedures adopted are given below:

5.6.2.1. Algal Content of the Biomass: The algal content was determined by methanol extraction method as indicated by McKinney (1941). The method is briefly given as follows: 10 ml of the mixed liquor was taken and centrifuged at 3000 rpm for 15 minutes. The pellet was taken and 10 ml of hot methanol was added and shaken well. Then the tube was put it warm water and

centrifuged and supernatant poured into a bottle. Again 10 ml of methanol was added and the contents put in a test-tube and heated in a beaker containing warm water. Again it was centrifuged and the supernatant poured into the bottle. This was repeated three times and the whole was made up to 50 ml. The optical density was found out in Spectronic-20 at a wave-length of 660 m and light path of 15 mm.

Standard curve was prepared by taking two known volumes of the mixed liquor and centrifuging the samples separately. One portion was dried and its weight found out. The other portion was treated with hot methanol and the absorbance found out at various dilutions. From this the dry weight and chlorophyll content were correlated. A typical standard curve is shown in Appendix B.

5.6.2.2. Alkalinity: As the pH in all the cases was below 8.3, the methyl orange alkalinity was determined as specified in Standard Methods (1965).

5.6.2.3. Biomass Content in the Effluent: The method was as follows. 500 ml of algal suspension was taken and divided into 2 equal parts. Each portion was centrifuged separately and the residue collected. The residue from one portion was dried and the dry weight found out. The other portion was diluted to various concentrations and the absorbance measured at a wave-length of 400 m and light path of 15 mm. A typical calibration curve is shown in Appendix B. From this graph the unknown biomass concentrations were determined by finding out the absorbance.

5.6.2.4. Calcium: Calcium was estimated by EDTA titrimetric method using murexide as indicator as per Standard Methods (1965).

5.6.2.5. Chemical Oxygen Demand (COD): COD analyses were conducted as per the method given in Standard Methods (1965). Sample volume taken was 20 ml or a small fraction diluted to 20 ml in all cases.

5.6.2.6. Nitrogen-Ammonia: Samples were taken and analysed by direct nesselerisation as per Standard Methods (1965). The wave-length used was 410 m with a light path of 15 mm. Standard curves were drawn for each set of reagents and checked at times. Analytical reagent quality NH_4Cl was used to prepare the standard curves. A typical standard curve is shown in Appendix B.

5.6.2.7. Nitrogen-Nitrate: Brucine method as given in Standard Methods (1965) was adopted in the determination of nitrate. A wave-length of 410 m at a light path of 15 mm was adopted. A typical calibration curve is shown in Appendix B.

5.6.2.8. Nitrogen-Nitrite: Phenol disulphonilic acid reagent method as per Standard Methods (1965) was adopted in determination of nitrite. A wave-length of 510 m at a light path of 15 mm was adopted. A typical standard curve is shown in Appendix B.

5.6.2.9. Nitrogen-Urea: A direct spectrophotometric analysis was adopted to find out the concentration of urea in a solution (Charbit, 1966). A sample volume of 3 ml was taken in which 1 ml of colouring reagent (8.5 g p-dimethyl amine benzaldehyde,

60 ml acetic acid, 10 ml hydrochloric acid and 32 ml distilled water) was added. The volume was made up to 5 ml with distilled water and absorbance was recorded at 440 m and light path of 15 mm after 10 minutes. A typical calibration curve is shown in Appendix B.

5.6.2.10. Nitrogen-Total: Total nitrogen is determined as per method stipulated by Stephensen (1977). The reagents required are: digestion solution, sodium hydroxide solution and nessler's reagent. Digestion solution was made by combining 40 g potassium sulphate, 250 ml water, 250 ml of concentrated sulphuric acid and 2 ml of selenium oxychloride in the order given. Sodium hydroxide solution was made by dissolving 500 g NaOH and 25 g of $Na_2S_2O_3$ in ammonia free distilled water and diluting to 1 l. Proportionate smaller quantities have been taken.

5 ml of the sample was put in the digestion flask and the required amount of digestion fluid was added as given in Table 11. A blank was run by putting 5 ml of distilled water in a flask and adding 1 ml of digestion fluid. Glass beads were put and digested for 2 hours till the contents became clear. Samples were diluted with ammonia free water and neutralised to pH 10 with NaOH solution. Precipitation by $ZnSO_4$ solution and centrifugation was done and the clear supernatant was nessel-erised and ammonia concentration found out, using Spectronic-20.

Table 11 - Digestion Solution and NaOH Solution to be Added for Total N Determination

Concentration of N in the sample, mg/l	Digestion fluid required, ml	NaOH solution required, ml
10000-5000	6-8	4-8
5000-2500	3-4	3-4
2500-500	2-3	2-3
500-50	1.0	1.0
Less than 50	0.5	0.5

5.6.2.11. pH: pH was measured by using the pH meter after calibrating the same with a buffer of known pH.

5.6.2.12. Phosphate: Sample preparation and digestion were done as per sulphuric acid-nitric acid method and analysis done as per stannous chloride method as per Standard Methods (1965).

5.6.2.13. Settling Value: Settling value was found by taking one litre of the mixed liquor in a 1 litre measuring cylinder and allowing the contents to settle for half an hour. The volume of settled sludge was measured. Such readings were taken at the end of every 2 days.

5.6.2.14. Suspended Solids: Membrane filter technique as outlined by Englebrecht et.al. (1956) was used to find out the mixed liquor biomass concentration. 10 ml samples were

used to find out the biomass concentration. The sample was filtered through 0.45 m millipore filter paper and dried in silica jel bottles and the dry weight found out.

5.6.3. Microscopic Examination:

Microscopic examination was done at various times during different seasons. A loopful of the mixed liquor was taken and observed in a microscope. The species present and the adherence of bacteria to algae were noted down.

6. RESULTS AND DISCUSSION

6.1. General:

This research was essentially divided into 3 phases, each with its own objective. Phase 1 was a series of batch studies in which all operating parameters such as biomass concentration, detention time, the effect of organic carbon on the operation, the effect of carbon dioxide on the performance, the effect of pH on ammonia removal and the effect of sewage on performance were involved. Besides these a comparative study of activated sludge system, algal system and flocculating algal-bacterial system was done. Feasibility study was done prior to the development of the optimum parameters.

Phase 2 study consisted of 3 batch reactors put in series. This study was conducted in order to obtain an effluent of

high standard as a single batch reactor was not capable of producing a high quality effluent. From this study the biomass concentrations required in Reactors 2 and 3 were also determined.

Phase 3 study was a continuous flow experiment where a working model reactor was constructed and operated with sludge recycling and also with various SRTs. Although 3 batch reactors in series were found to work much better than a single batch reactor, such an arrangement was not possible in the laboratory for the continuous flow study. Hence a single reactor was used in this study.

Biokinetic constants were evolved from the continuous flow study. The experiments in all the 3 phases were conducted in sunlight with no temperature control. This was done such that the experimental results could be translated into a pilot plant study in the field.

6.2.1. Phase 1 - Part 1: Feasibility Study:

The algal-bacterial biomass developed as explained in Section 5.2.3 was used in the feasibility study. Full details of experimental procedure are given in Section 5.3.1. The operating conditions are summarised in Table 12.

Table 13 gives the complete data regarding biomass, algae/bacteria ratio, total N removal efficiency and COD removal efficiency through 18 days in this experiment, the operating parameters of which were already given in Table 12.

Table 12 - Operating Conditions in Phase 1 - Part 1 Study, Temperature 33-41°C

Reactor	NH_3-N dose mg/l	COD dose mg/l	Phosphate dose mg/l	Step input of NH_3-N upto 12th day, mg/l
1	250	1840	140	100-150-200-250
2	975	7340	560	300-500-675-975
3	975	7540	560	400-575-750-975
4	1000	7450	560	500-650-950-1000

The biomass was acclimatised for the first 12 days and thereafter no feed (ammonia, glucose and phosphate) was given. However, the biomass growth was rather rapid in Reactors 2, 3 and 4, where the initial ammonia doses were 300, 400 and 500 mg/l respectively. The ammonia N dose at the 12th day in Reactor 1 was 250 mg/l; the corresponding doses in other reactors were 1000 mg/l each. This study indicated that even an initial dose of 500 mg/l of ammonia N at a pH of 7.9 did not have any deleterious effect on 1400 mg/l of algal-bacterial biomass.

The biomass concentrations attained on the 12th day were 4000, 5800, 5200 and 5600 mg/l in Reactors 1, 2, 3 and 4 respectively. The nutrients were utilised rapidly in Reactors 2, 3 and 4. So in a nutrient depleted condition the biomass concentrations had started decreasing. However, in Reactor 1

Table 13 - Biomass, Algae/Bacteria Ratios, Total N and COD Removal Efficiencies

Days \ Reactors	Biomass concentration, mg/l				Algae/bacteria ratio				Total N removal efficiency, percent				COD removal efficiency, percent			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
0	1400	1400	1400	1400	1.54	1.54	1.54	1.54								
2	1600	1400	1100	1200					75	58	69	70	95	95	96	96
4	2000	1300	1400	1400	1.50	1.22	1.64	1.33	75	75	67	70	95	95	95	96
6	2100	1900	2800	2700					70	69	77	65	100	96	96	96
8	2400	2700	3800	3700	1.40	1.08	1.38	1.18	65	74	72	68	100	96	94	95
10	2800	4600	3800	4800					78	77	67	68	93	97	95	97
12	4000	5800	5200	5600	1.22	1.32	1.36	1.95	68	76	72	73	94	96	94	95
14	4600	7400	7600	7800					70	71	71	74	94	98	96	96
16	5400	7000	6900	7400	1.45	1.33	1.30	1.46	74	68	71	67	94	98	96	96
18	6600	6600	6700	6900	1.27	1.27	1.31	1.38	74	68	70	69	94	98	96	96

this phenomenon was taking place comparatively at a slow rate. This could be one of the advantages of high initial and high step feed of ammonia N.

The biomass concentration reached a maximum value of 6600 mg/l in Reactor 1 on the 18th day. The maximum biomass concentrations in Reactors 2, 3 and 4 were 7400, 7600 and 7800 mg/l respectively. After 14th day the biomass concentrations were on the decline. This is indicated in Figure 13.

Algae/bacteria ratios were fluctuating in a narrow range of 1.22-1.45 in all the reactors except in Reactor 4. This variation is shown in Fig. 14. This indicates that the above ratio was more or less steady for 6 days after acclimatisation.

The quantities of nitrite N and nitrate N were negligible. The values were of the order of 12-16 mg/l in all reactors. This might have happened due to the presence of organic carbon, free ammonia (NH_3) and exposure to sunlight (Anthonisen et.al., 1976). Moreover, nitrifiers being autotrophic and more sensitive to environmental conditions, might not have withstood the competition for CO_2 against algae. Hence the presence of insignificant quantities of oxidised nitrogen.

Alkalinity drop was sudden in 2 days after acclimatisation. It dropped from an initial concentration of 510 mg/l to a final value of 120-140 mg/l in all reactors. This might be due to the extraction of CO_2 from alkalinity for algal growth. However, at no time the alkalinity was found to fall below

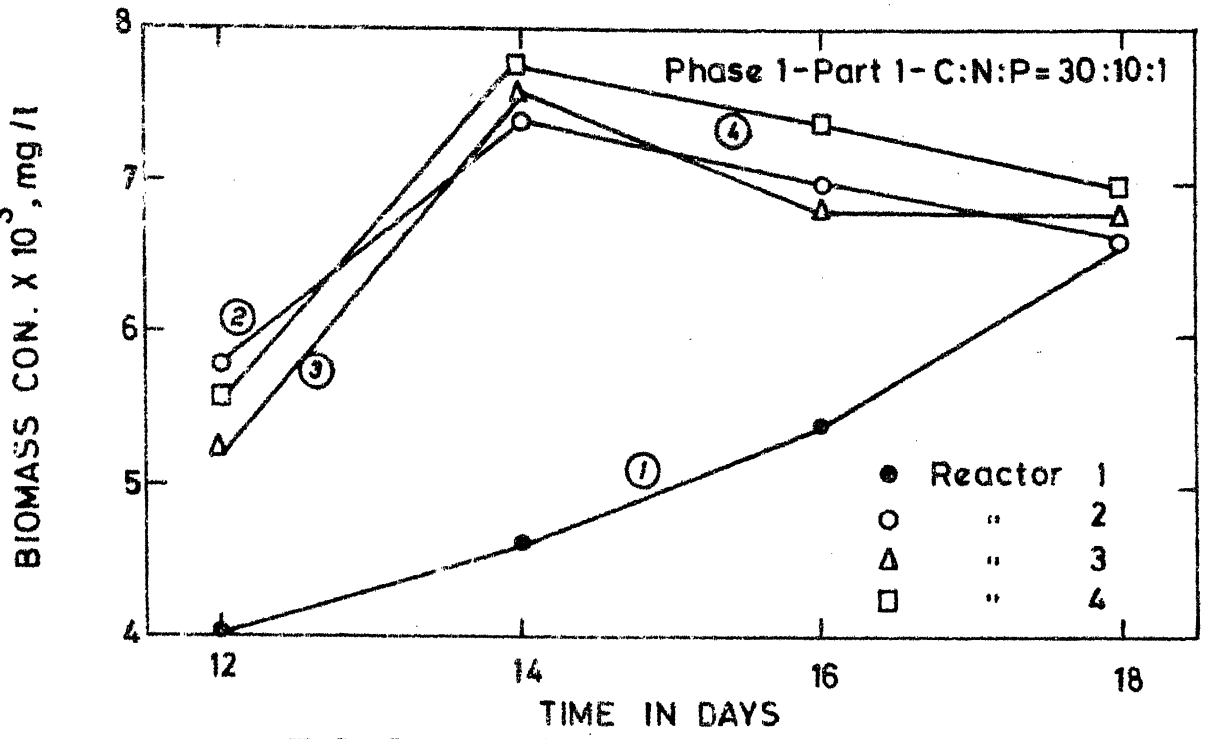


FIG.13 BIOMASS GROWTH VS. TIME

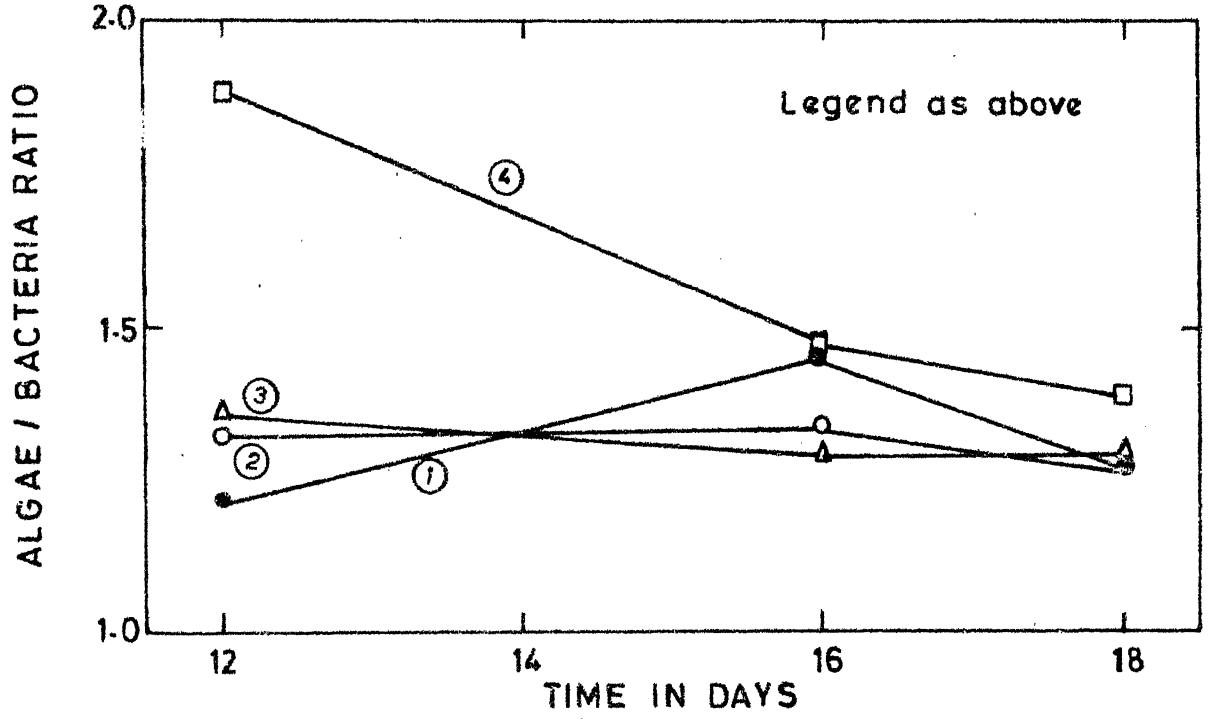


FIG.14 ALGAE / BACTERIA RATIO VS. TIME

120 mg/l. This might be due to the continuous replenishment of the H_2CO_3-HCO_3^--$CO_3^=$ system. The CO_2 for the replenishment would have come from the biomass respiration and continuous aeration.

The total nitrogen removal efficiency after acclimatisation is shown in Figure 15. The efficiencies were 70-74, 61-71, 70-71 and 67-74 percent in Reactors 1, 2, 3 and 4 respectively. The total N removal efficiency remained more or less the same irrespective of the ammonia feed pattern during acclimatisation.

COD removal efficiency ranged from 94-100 percent in all reactors. This is shown in Figure 16. After acclimatisation the COD removal efficiencies were 94, 98, 96 and 96 percent in Reactors 1, 2, 3 and 4 respectively. It indicates that almost full utilisation of organic matter was achieved.

pH was decreasing from 7.9 on the 12th day to 6.7 on the 18th day in Reactors 1 and 2 and from 7.9 to 6.2 in Reactors 3 and 4.

From these facts it was found that flocculating algal-bacterial system was capable of removing ammonia from high nitrogenous wastes. Even an initial biomass concentration of 1400 mg/l could withstand an ammonia N concentration of 500 mg/l at a pH 7.9. Moreover, it was found that at a C:N:P ratio of 30:10:1 and a maximum feed of ammonia N of 1000 mg/l, a biomass concentration of 7800 mg/l could be attained. So Part 2 of Phase 1 was started in order to find out the ammonia N removal pattern with lesser quantities of organic carbon.

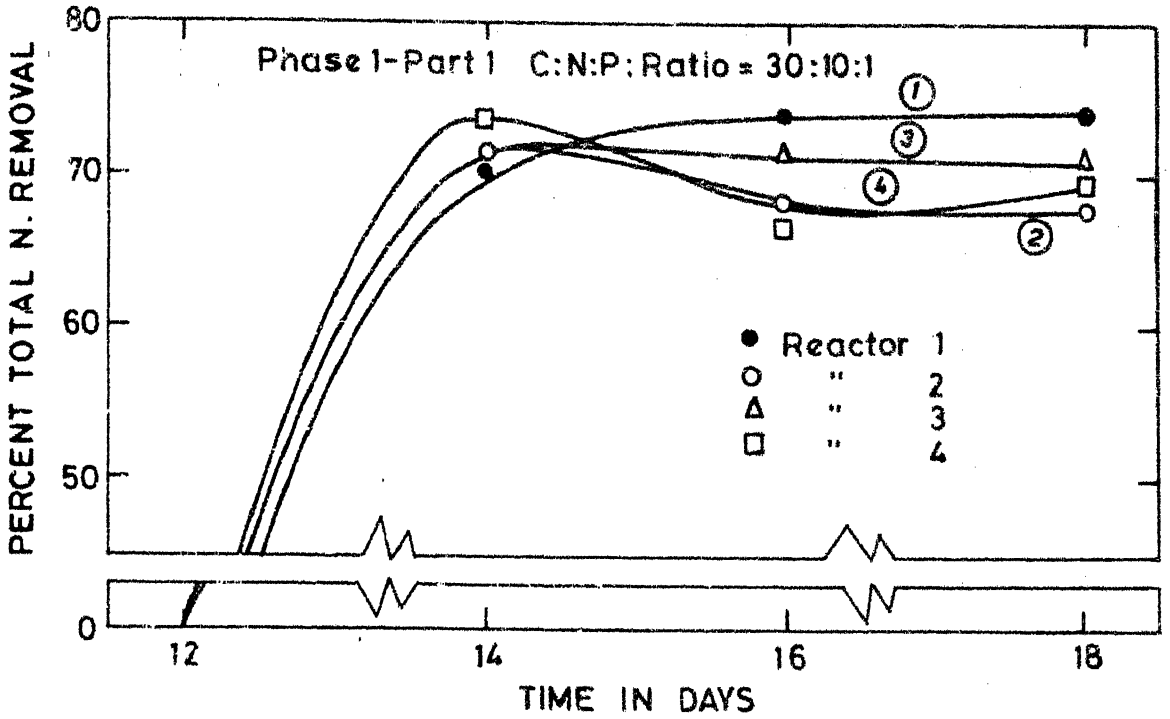


FIG.15 TOTAL NITROGEN REMOVAL VS. TIME

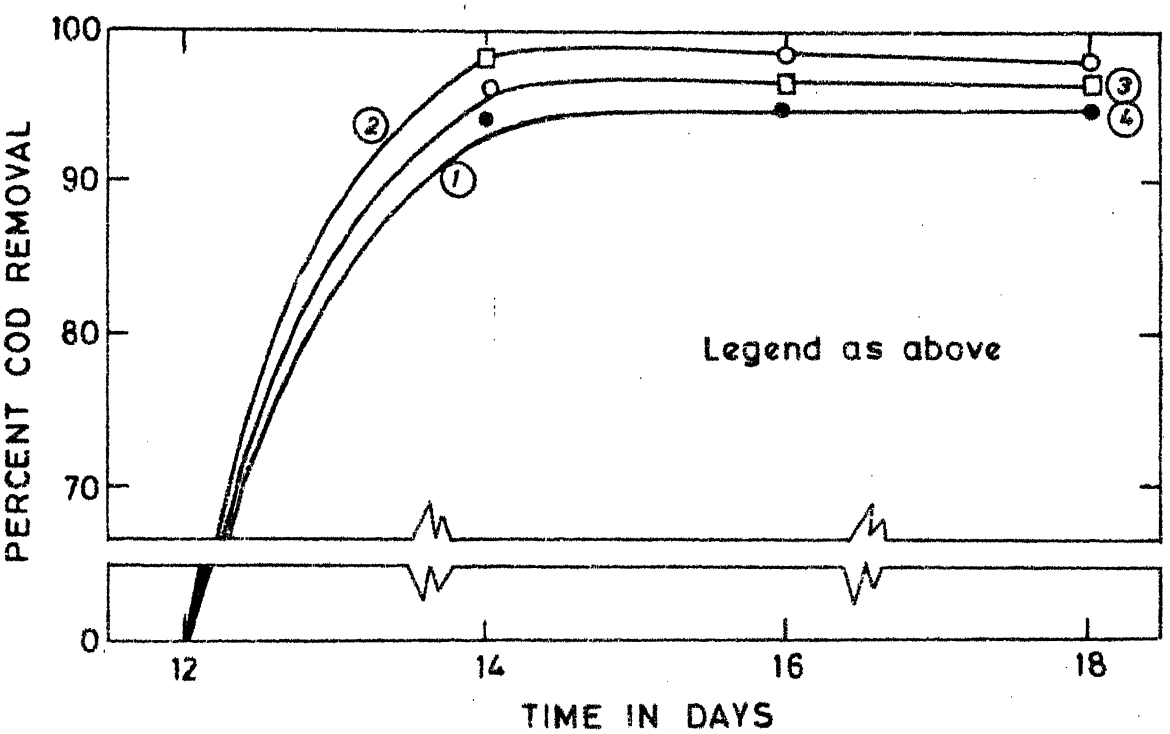


FIG.16 COD REMOVAL VS. TIME

6.2.2. Phase 1 - Part 2: Ammonia Removal Study with C:N:P Ratios of 30:10:1, 25:10:1, 20:10:1 and 15:10:1:

The details of the experimental study are given in Section 5.3.2. The operating conditions are summarised in Table 14.

Table 14 - Operating Conditions in Phase 1 - Part 2 Study, Temperature 29-34 °C

Reactor	C:N:P ratio	Ammonia N dose mg/l	Organic carbon-COD mg/l
1	30:10:1	975	7490
2	25:10:1	950	6340
3	20:10:1	950	5180
4	15:10:1	950	3650

This experiment was conducted with the biomass, which was already acclimatised in the experiment in Phase 1 - Part 1. The biomass was properly washed before it was used for the experiment. The following Table 15 shows the details pertaining to the days after acclimatisation and stopping of the feed.

The biomass growth was rather high during 2 days in Reactor 1. From an initial biomass concentration of 7000 mg/l, it rose to 10000 mg/l during that period. The pattern of growth is illustrated in Figure 17. However, the biomass growth in other reactors during the first 2 days was proportional to the organic

Table 15 - Biomass, Algae/Bacteria Ratios, Total N and COD Removal Efficiencies

Reactors / Days	Biomass concentration, mg/l				Algae/bacteria ratio				Total N removal efficiency, percent				COD removal efficiency, percent			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
0	7000	5600	5600	5000	1.10	1.24	1.54	1.77								
2	10000	8200	6400	6000	0.96	1.22	1.46	1.73	75	73	70	72	96	94	95	95
4	7200	5600	5400	5000					77	81	77	77	97	95	96	95
6	7500	6200	7500	4200	0.92	1.06	1.58	1.80	80	82	79	82	97	96	96	96
8	7600	7100	6200	4400					80	81	79	79	98	97	97	96
10	6600	5800	6000	4100	0.82	1.32	1.50	2.15	82	82	81	79	98	97	97	97

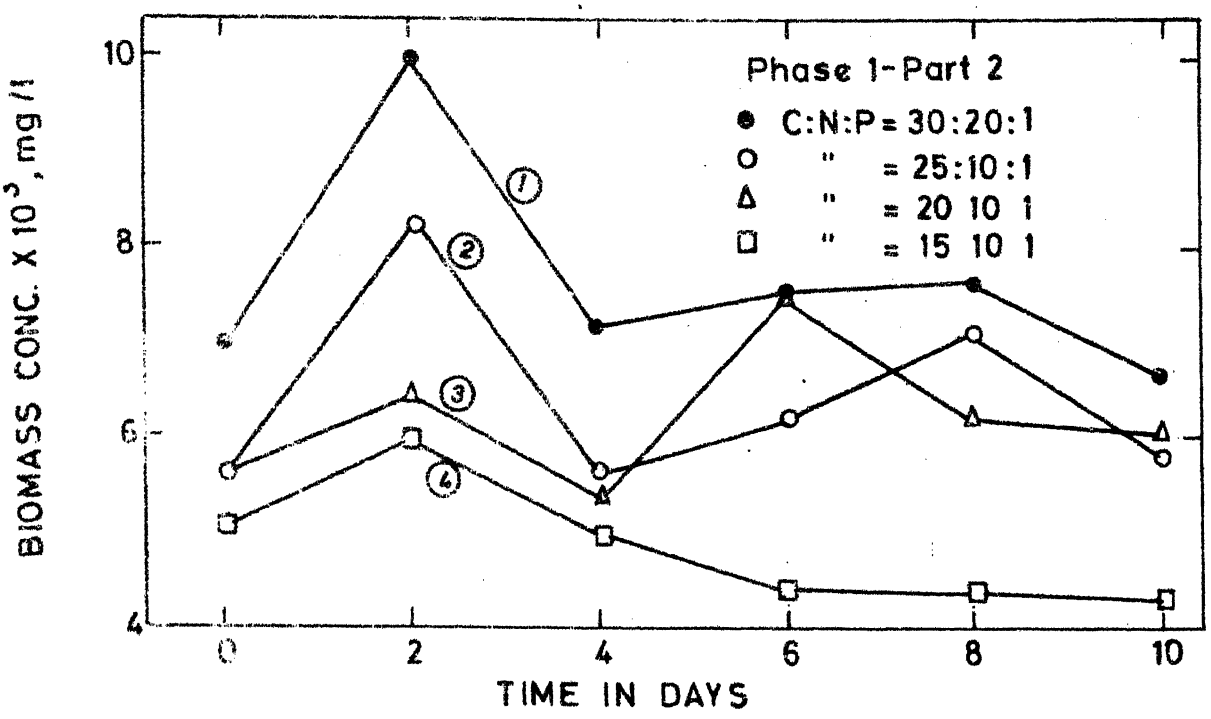


FIG.17 BIOMASS GROWTH VS. TIME

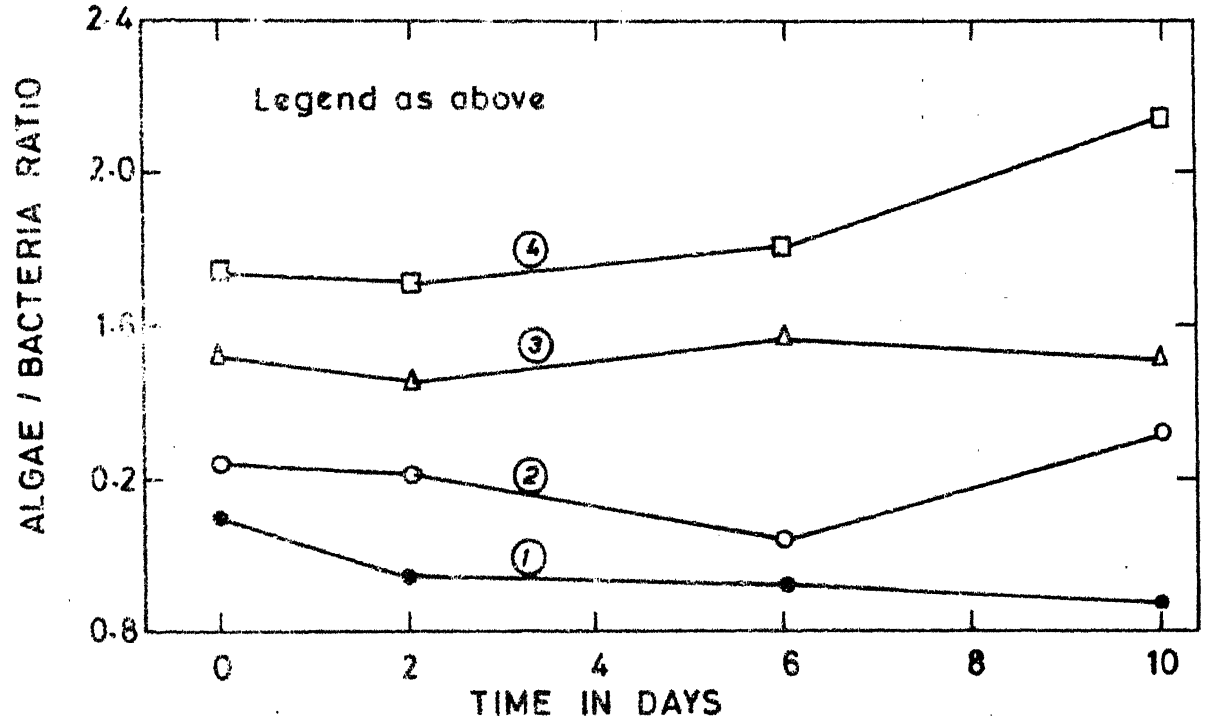


FIG.18 ALGAE / BACTERIA RATIO VS. TIME

carbon content of the feed. The biomass concentration in all the reactors declined after 2 days. This decline might be due to the decline in the bacterial biomass. As the C:N:P ratio increased, the algae/bacteria ratio decreased. This is clear from Figure 18. Organic carbon limitation might be the cause for the phenomenon. Further details are presented in the data in Appendix C.2.

The trend of total N removal efficiency is shown in Figure 19. The total N removal efficiency was about 80 percent on the 8th day. The total N removal efficiency was somewhat higher (77 percent) for Reactors 1 and 2 as compared to that (71 percent) in Reactors 3 and 4 during first 4 days. Yet another feature noted was that in spite of biomass reduction, the total N removal efficiency did not decrease. This might be due to incorporation of more than the stoichiometric amount of N in the biomass. This was studied in greater detail in Phase 1 - Part 7.

pH drop was continuous. From an initial pH of 8.1-8.3 it reached a value of 6.4-6.6 in all reactors. This might be mainly due to the extraction of ammonia N and release of H^+ ions.

The sludge was rather fluffy in Reactor 1. However, those in Reactors 2, 3 and 4 were comparatively more compact. The lesser the organic carbon content the more compact the sludge was.

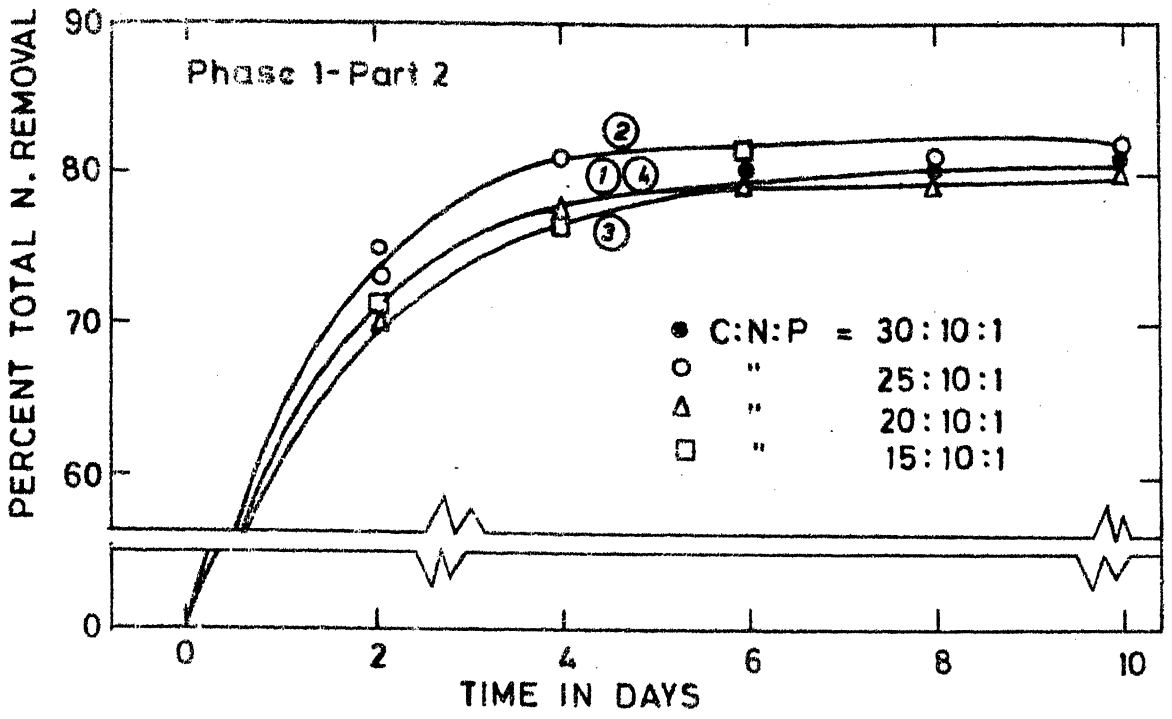


FIG. 19 TOTAL NITROGEN REMOVAL VS. TIME

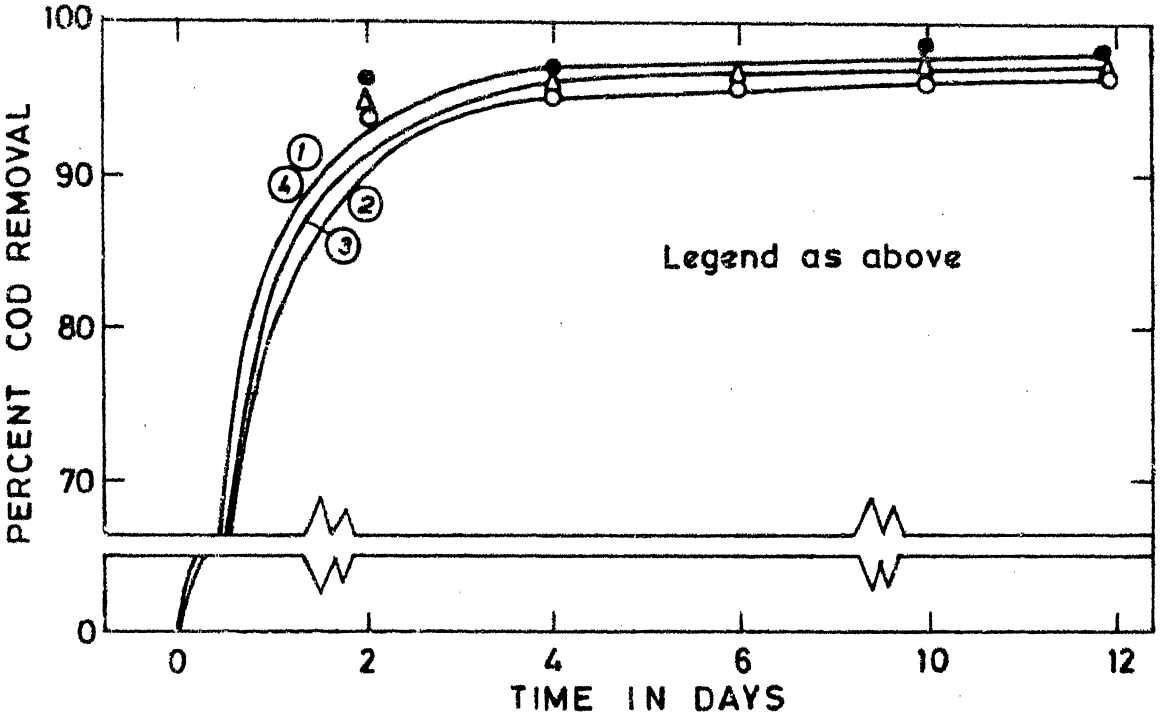


FIG. 20 COD REMOVAL VS. TIME

The supernatant contained biomass to the extent of 70-80 mg/l. In reactors where organic carbon was more, the supernatant was more clear. As the detention times increased, algal contents in the supernatant also increased. This might be due to the growth of new algal cells which tend to float rather than flocculate and settle down. So it was observed that in batch studies a detention period of 2 days would be ideal. Total N and COD removal efficiencies were not increasing significantly after 2 days.

The COD removal pattern is shown in Figure 20. The COD removal efficiency was around 95-97 percent in all the reactors. A small quantity of organic carbon, which might be algal-bacterial excretions/lysed cells, might be the source of residual COD observed in the filtrate.

As a C:N:P ratio of 20:10:1 was able to sustain a biomass concentration of 5000-6000 mg/l during the first 4 days with about 72-77 percent of total N removal efficiency, it was decided to study the effect of nitrogen removal efficiency with 1 percent CO_2 supplement at C:N:P ratios of 20:10:1 and 15:10:1.

6.2.3. Phase 1 - Part 3: Ammonia Removal Study with C:N:P Ratios of 20:10:1 and 15:10:1 with and without Carbon Dioxide:

The operating conditions in this part of the Phase 1 study are presented in Table 16. The biomass from the Phase 1 - Part 2 was used in this experiment with proper washing and

Table 16 - Operating Conditions in Phase 1 - Part 3 Study, Temperature 24-31°C

Reactor	C:N:P ratio	1 percent CO_2 addition	Ammonia N dose, mg/l	Organic carbon-COD mg/l
1	20:10:1	No	950	5290
2	15:10:1	No	975	3530
3	20:10:1	Yes	950	5100
4	15:10:1	Yes	950	3530

acclimatisation as described in Section 5.3.3. All details are presented in Table 17.

Concentration of CO_2 (1 percent) was chosen on the basis of values cited in the literature (Myers, 1953; Golueke et.al., 1959; Oswald, 1963).

The biomass growth pattern is illustrated in Figure 21. There was little reduction of biomass in Reactor 1, whereas there was gradual drop in the biomass concentration in Reactor 2. This was possibly due to organic carbon limitation. However, the biomass growth was more in Reactor 3 than that in Reactor 1 as CO_2 might have aided in the process.

Algae/bacteria ratio was of the order of 1.8-2.0 in Reactor 3 whereas it was 1.40-1.70 in Reactor 1. This is shown in Figure 22. There was continuous growth of biomass with algae/bacteria ratio ranging from 1.5-2.5 in Reactor 4. Algae was

Table 17 - Biomass, Algae/Bacteria Ratios, Total N and COD Removal Efficiencies

Days \ Reactors	Biomass concentration, mg/l				Algae/bacteria ratio				Total N removal efficiency, percent				COD removal efficiency, percent			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
0	5200	6000	5000	5600	1.78	1.34	1.77	1.80								
2	6000	5600	6200	6000	1.50	1.54	1.82	1.50	56	57	61	63	92	94	92	93
4	5400	5400	6000	6400	1.70	1.70	2.00	1.67	61	61	63	63	92	95	92	95
6	6000	4800	6400	6200	1.40	1.20	1.92	1.80	62	61	63	60	92	95	93	95
8	6200	5000	7000	6600	1.40	1.27	1.80	2.30	62	61	61	65	93	95	91	96
10	6000	4000	6000	6400	1.40	1.35	2.00	2.50	44	43	41	40	91	93	91	93

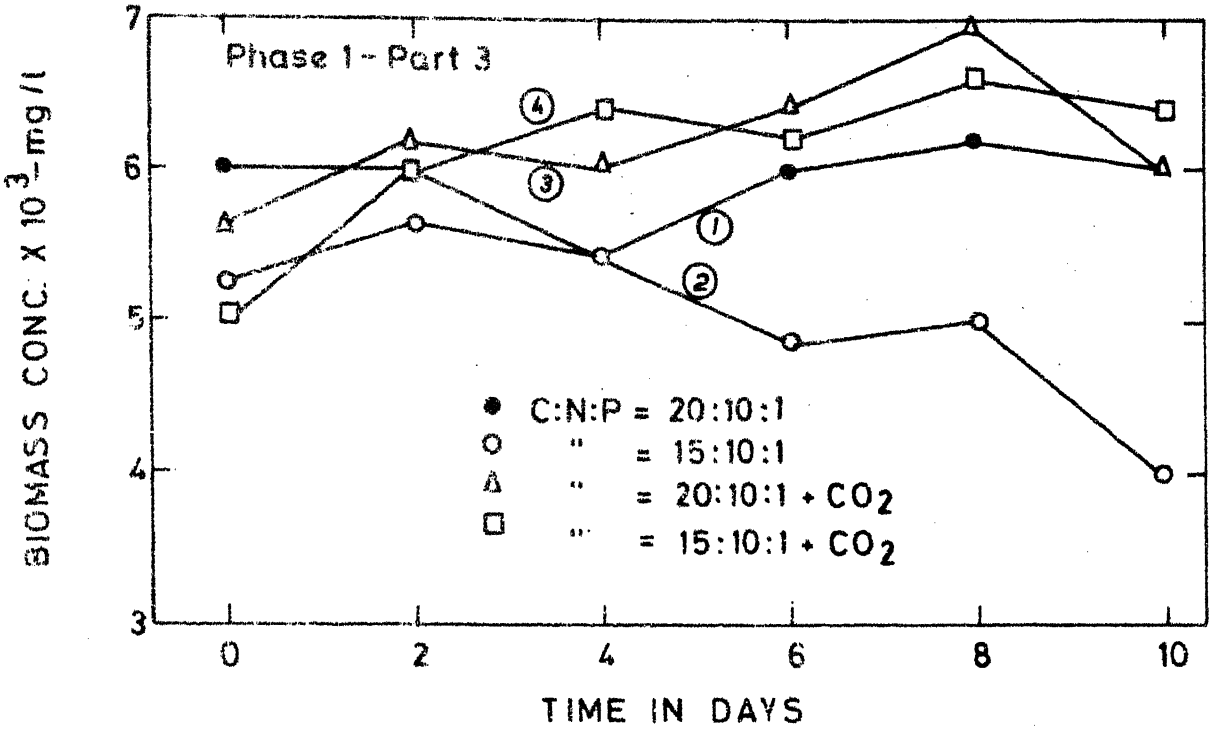


FIG. 21 BIOMASS GROWTH VS. TIME.

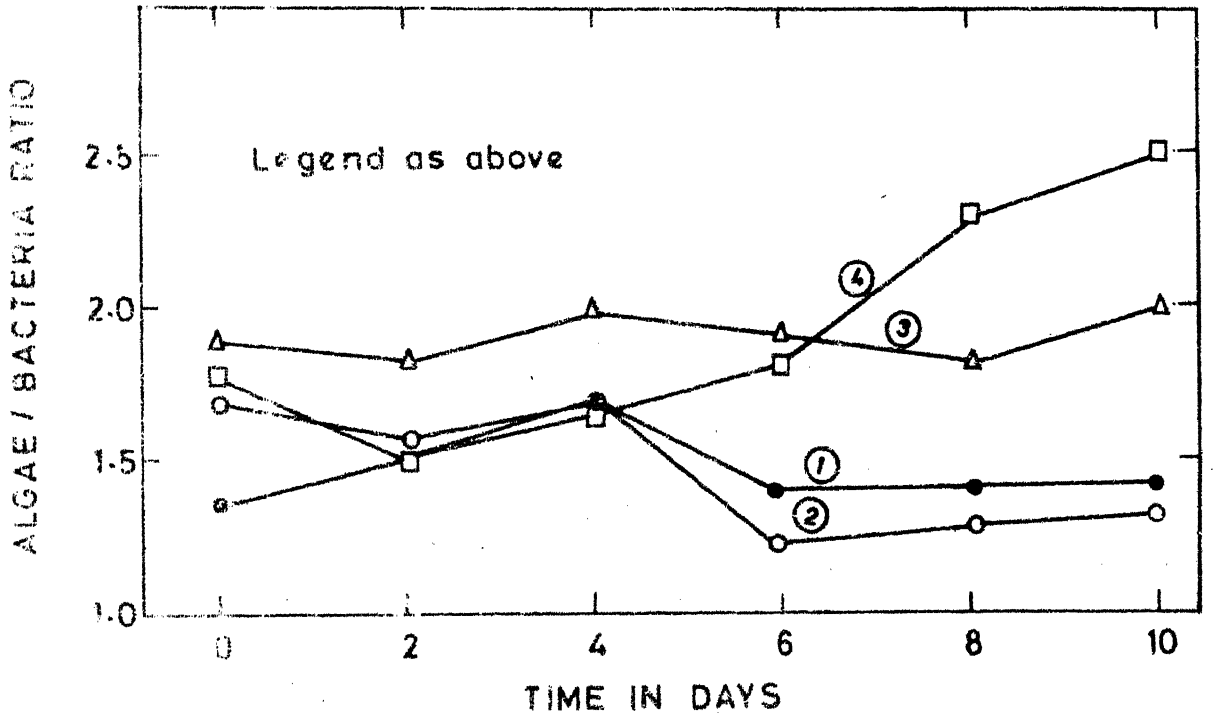


FIG. 22 ALGAE / BACTERIA RATIO VS. TIME

found to be more in quantity in this reactor, due to the effect of CO_2 supplement.

Total N removal efficiency ranged from 56-62 percent during 8 days in Reactor 1. It is shown in Figure 23. The same was found to be 57-61 percent in Reactor 2, 61-63 percent in Reactor 3 and 60-65 percent in Reactor 4. The percentages were found to be of the order of 43-44 percent on the 10th day in all reactors. This was closely followed by increase in the COD of the effluent. The COD removal efficiency pattern is shown in Figure 24. The total N removal efficiency did not appreciably increase depending on the C:N:P ratio or CO_2 supplement in the range studied.

pH reduction was less gradual from 2nd day onwards in all reactors. Alkalinity was following the same trend as in the earlier experiments.

Although there was biomass increase in Reactors 3 and 4, there was little enhancement in the total N removal efficiency. So CO_2 addition did not significantly improve the total N removal efficiency. At longer detention times the effluent contained more and more of biomass. This is illustrated in Table 18. This might be due to lysis of cells or breaking up of flocs. Also the values of settled sludge increased in Reactors 3 and 4. However, the settling values in Reactors 1 and 2 were 80-100 ml. So it was inferred that CO_2 was not conducive for proper functioning of the system.

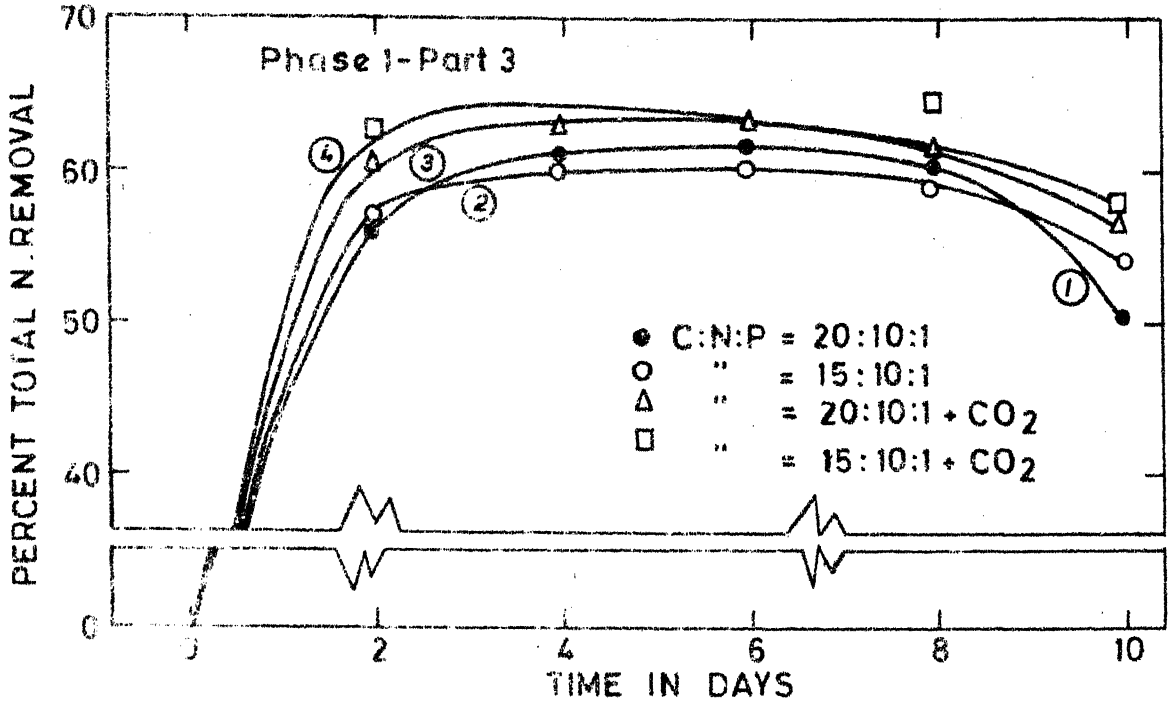


FIG. 23 TOTAL NITROGEN REMOVAL VS. TIME

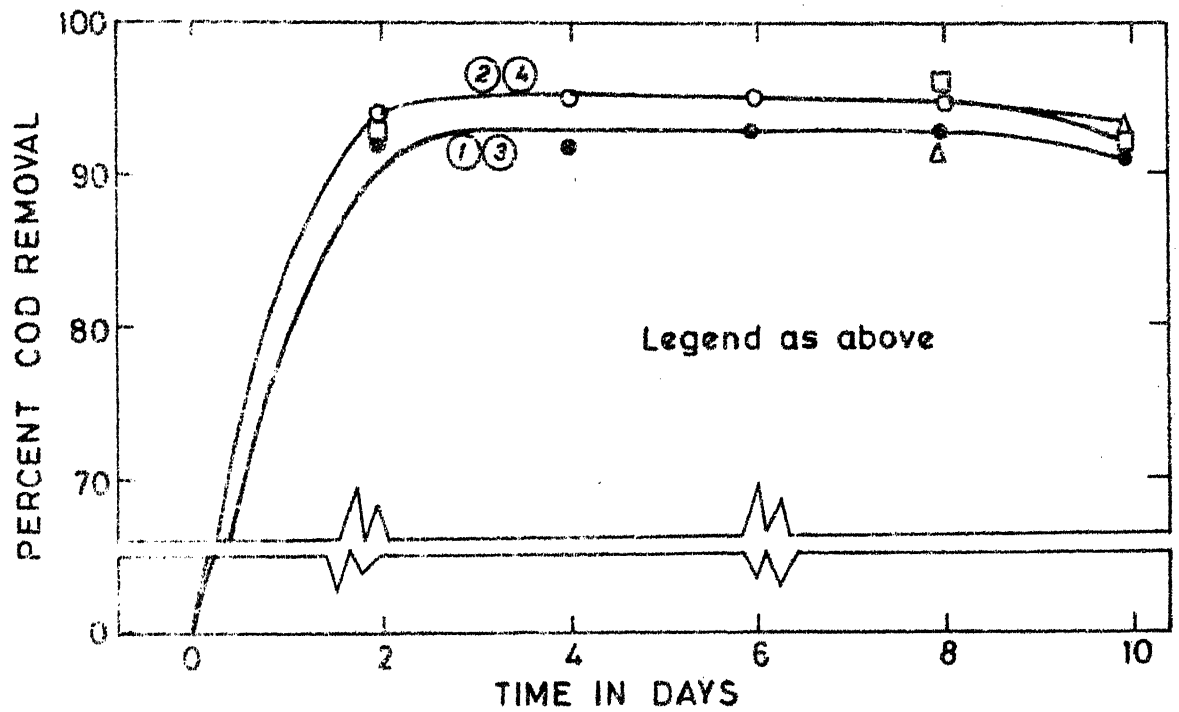


FIG. 24 COD REMOVAL VS. TIME

Table 18 - Settling Values and Effluent Biomass Concentrations

Reactor \ Day	Settling values ml					Effluent biomass concentration, mg/l			
	0	3	5	7	10	0	2	8	10
1	100	100	100	90	80	100	100	130	100
2	120	120	120	110	100	130	130	160	130
3	140	140	150	160	140	130	180	130	160
4	140	140	160	160	160	160	180	180	180

Even the organic carbon as in C:N:P ratio of 15:10:1 could sustain viable and functioning biomass. Probably, still lower organic carbon content may be capable of sustaining the biomass. This experiment indicated that the shorter the detention time, the better the performance of reactors.

It might be due to endogenous respiration and lysis of cells that ammonia was released in the reactor contents on the 10th day. Within the range studied, the study indicated that algae/bacteria ratio had little effect on total N removal efficiency at pH lower than 7.0. However, the settling property and effluent biomass concentrations were of acceptable standard with larger organic carbon contents. This is an important factor to be taken into consideration.

6.2.4. Phase 1 - Part 4: Ammonia N Removal with C:N:P Ratios of 14:10:1, 6:10:1 and 3:10:1 and Sewage:

With a view to reduce the organic carbon dose as much as possible, it was decided to conduct experiments with C:N:P ratios of 14:10:1, 6:10:1, 3:10:1 and sewage with C:N:P ratio of 3:10:1. The operating conditions of this experiment are summarised in Table 19.

Table 19 - Operating Conditions in Phase 1 - Part 4 Study, Temperature 12-19°C

Reactor	C:N:P ratio	Sewage addition	Ammonia N dose, mg/l	Organic carbon-COD mg/l
1	14:10:1	No	1050	3460
2	6:10:1	No	950	1550
3	3:10:1	No	950	770
4	3:10:1	Yes	950	810

The C:N:P ratio of 3:10:1 for feed in Reactor 4 was arrived at, taking into consideration of the COD in the sewage. Sewage was not tried along with the higher ratios of C:N:P, as the COD was of the order of 190 mg/l.

The biomass growth pattern is shown in Figure 25. The results obtained are presented in Table 20. The initial biomass concentration was about 4000 mg/l in all reactors. The algae/bacteria ratio at the beginning was 1.5. During acclimatisation, the

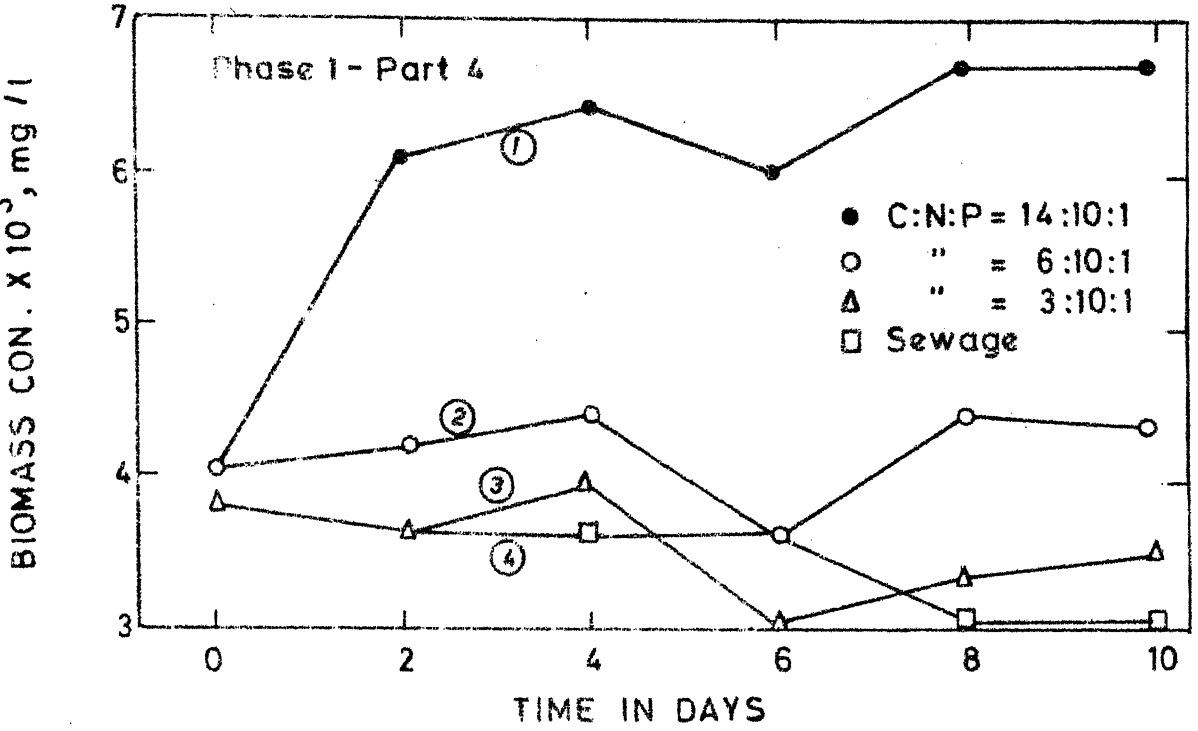


FIG. 25 BIOMASS GROWTH VS. TIME

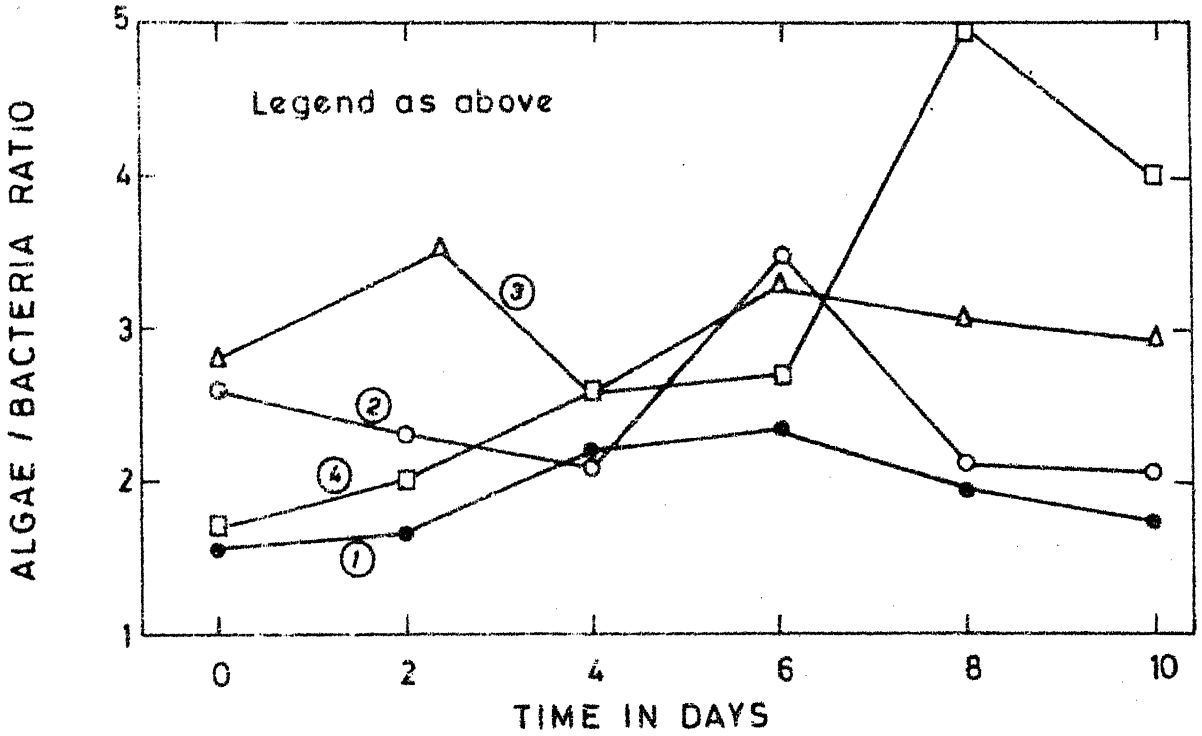


FIG. 26 ALGAE / BACTERIA RATIO VS. TIME

Table 20 - Biomass, Algae/Bacteria Ratios, Total N and COD Removal Efficiencies

Reactors / Days	Biomass concentration, mg/l				Algae/bacteria ratio				Total N removal efficiency, percent				COD removal efficiency, percent			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
0	4000	4000	3800	3800	1.57	2.60	2.80	1.70								
2	6100	4200	3600	3600	1.65	2.30	3.50	2.00	35	30	27	28	98	95	100	100
4	6400	4400	3900	3600	2.20	2.14	2.50	2.60	37	33	30	50	98	95	95	91
6	6000	3600	3000	3600	2.33	3.50	3.30	2.70	37	28	27	27	98	93	85	91
8	6700	4400	3300	3000	1.90	2.14	3.10	5.00	41	38	31	30	98	95	90	91
10	6700	4300	3500	3000	1.70	2.10	2.90	6.00	41	33	30	30	99	95	95	100

biomass development was different in the various reactors. After the feed was given, the biomass was increasing through 10 days in Reactor 1. The biomass concentration varied from an initial concentration of 4000 mg/l to 4400 mg/l on the 4th day. The algal biomass concentration was 4400 mg/l in Reactor 1, whereas it was never greater than 3000 mg/l in Reactor 2. The biomass was continuously declining from an initial concentration of 3800 mg/l in Reactor 3. So a C:N:P ratio of 3:10:1 was found to be insufficient for a balanced growth of the biomass. The biomass was declining from an initial value of 3800 mg/l to 3000 mg/l on the 10th day in Reactor 4. However, the algal biomass in the reactor was more or less constant throughout.

The pattern of variations of algae/bacteria ratios is shown in Figure 26. The ratio went from 1.57-2.50 in Reactor 1, from 2.1-3.5 in Reactor 2, from 2.5-3.5 in Reactor 3 and from 1.7-5.0 in Reactor 4. The higher the C:N:P ratio the lower was the algae/bacteria ratio. It can also be seen that the higher the C:N:P ratio the higher was the biomass concentration.

The total N removal efficiency for the reactors is as shown in Figure 27. The efficiencies were 35-41 percent in Reactor 1, 30-38 percent in Reactor 2, 27-31 percent in Reactor 2 and 28-30 percent in Reactor 4. These results indicated that the total N removal efficiency did not increase significantly with longer detention times. So a shorter detention time is

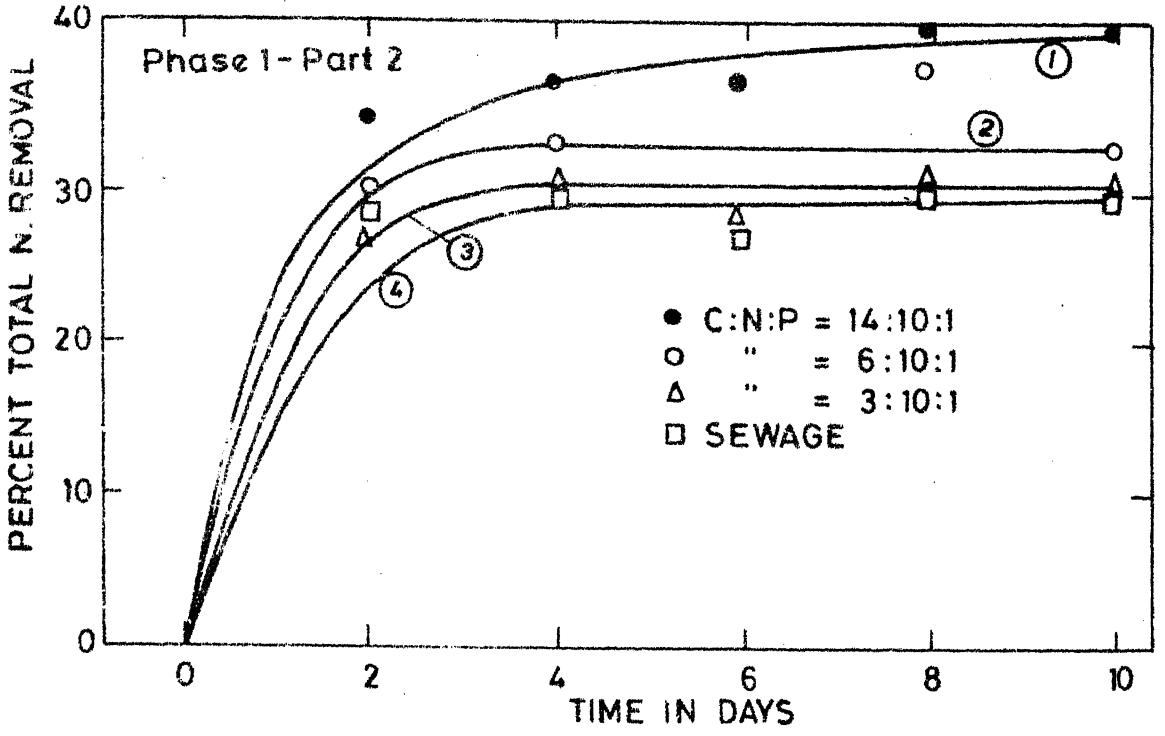


FIG. 27 TOTAL NITROGEN REMOVAL VS. TIME

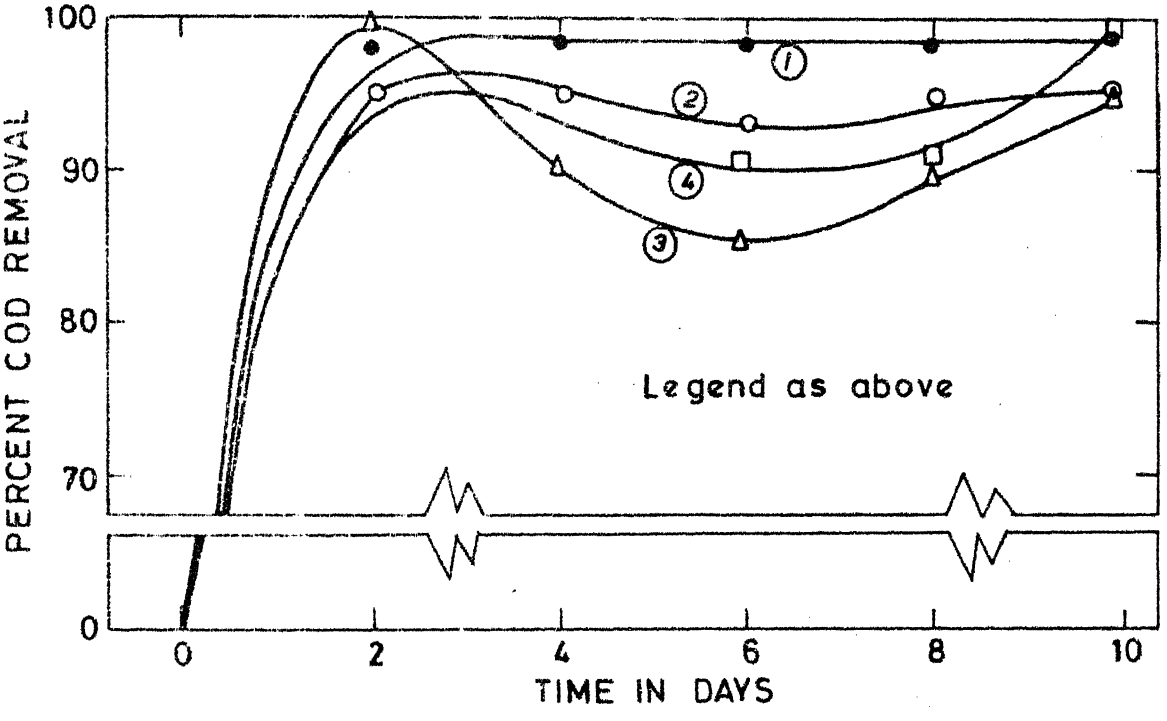


FIG. 28 COD REMOVAL VS. TIME

preferable. There was little difference in total N removal efficiencies among Reactors 2, 3 and 4. The efficiencies were about 30 percent in all the above reactors. However, the efficiency in Reactor 1 was 35-41 percent.

COD removal efficiency was about 98-99 percent in Reactor 1. This is shown in Figure 28. However, the efficiency was 95 percent in Reactor 2. The effluent COD in Reactors 3 and 4 on the 2nd day was zero. Then the effluent COD value increased. This increase might be due to the excretional products and lysed cells.

Table 21 shows the effluent characteristics in terms of biomass concentration and also the settling values of the sludge.

Table 21 - Settling Values and Effluent Biomass Concentrations

Days / Reactor	Settling values ml						Effluent biomass concentration, mg/l					
	0	2	4	6	8	10	0	2	4	6	8	10
1	140	120	120	120	120	120	65	65	90	150	150	150
2	140	130	120	100	100	100	80	100	65	210	180	200
3	120	120	110	80	80	80	60	90	60	210	200	210
4	120	120	110	90	90	90	110	100	80	210	210	210

It is evident from Table 21 that it would be preferable to have a C:N:P ratio between 14:10:1 and 6:10:1 from the point of view

of effluent biomass concentration and settleability of the biomass. Settling values improved with longer detention times in Reactors 3 and 4. However, there was more of biomass in the effluent. The effluent biomass concentrations were 65, 80, 60 and 110 mg/l in Reactors 1, 2, 3 and 4 respectively on the 2nd day. The values were 160, 200, 210 and 220 mg/l in Reactors 1, 2, 3 and 4 respectively on the 10th day.

pH dropped from an initial value of 7.8-7.9 to a final value of 5.6 in Reactor 1 and 6.9-7.0 in other reactors. pH rose up from 6.0 on the 6th day to 6.9-7.0 on the 10th day in Reactors 2, 3 and 4. It might be possibly due to the shift in the H_2CO_3-HCO_3^--$CO_3^=$ system with no interference from ammonia.

Alkalinity never dropped below 60-80 mg/l. It was rapidly falling from an initial value of 510 mg/l to 100 mg/l in 2 days. The same pattern was noted in earlier experiments too.

Sewage addition had little effect; neither on the total N removal efficiency nor on the biomass growth. In general, it was observed that the longer the detention time in a batch reactor, the lesser the COD removal efficiency.

Nitrite N and nitrate N ranged from 22-34 mg/l and 8-12 mg/l respectively in all reactors. Significant variations in nitrites and nitrates were not observed in any of the reactors. The effect of organic carbon on the nitrite or nitrate formation was not very much evident from this experiment. However, in an aerated system as this, the oxidised forms of

nitrogen never rose beyond 40-50 mg/l. This might be due to the competition, the nitrifiers have to face with algae with regard to inorganic carbon sources (Adams et.al., 1977). The growth of nitrifiers might have been suppressed due to exposure to sunlight (Anthonisen et.al., 1976).

pH in all reactors dropped down to 6.1-6.4 in 2 days. This was coinciding with the tapering off of total N removal efficiency. So it was decided to study the effect of pH on the total N removal efficiency.

6.2.5. Phase 1 - Part 5: Ammonia Removal Study with C:N:P Ratio of 6:10:1 with pH at 8.0-8.3:

In this section the pH was maintained at a range of 8.0-8.3 either by addition of Na_2CO_3 or by $Ca(OH)_2$. Reactor 1 was kept as a control with no pH manipulation. The operating conditions in this study are summarised in Table 22.

Table 22 - Operating Conditions in Phase 1 - Part 5 Study, Temperature 8-18°C

Reactor	pH control 8.0-8.3	Ammonia N dose, mg/l	Organic carbon-COD mg/l
1	No control	975	1550
2	Na_2CO_3	975	1510
3	$Ca(OH)_2$	975	1580

Significant penetration of ammonia starts only at pH 8.0 and above (Abeliovitch et.al., 1976). According to Warren (1962), uptake and assimilation of ammonia is dependent upon pH. Dohler (1971) reported that ammonia diffuses positively into the cells. Toxicity due to ammonia could be reduced by aeration (Loehr, 1974). Hence this experiment was conducted to study the total N removal efficiency at a pH range of 8.0-8.3. The results are tabulated in Table 23.

It could be seen from Figure 29 that there was increase in biomass growth in Reactors 1 and 2 upto 4th day. However, the biomass concentration was on the decline in Reactor 3 after 2 days. Thereafter there was a decline of biomass throughout in all the reactors.

Table 24 shows the algal biomass and bacterial biomass at various detention times. The maximum biomass concentrations attained were 5000 mg/l each in Reactors 2 and 3. However, the maximum biomass concentration was 6800 mg/l in Reactor 1. The variation of algae/bacteria ratio is shown in Figure 30.

Algal component in Reactor 1 was more compared to that in Reactors 2 and 3 at any time. This might be possibly due to high pH in Reactors 2 and 3. Flow of inorganic carbon into algal cells at pH 8.0-8.3 might have been rather slow (Giese, 1973). However, at pH of 6.6 in Reactor 1, inorganic carbon might have passed freely into algal cells and hence the high biomass concentrations.

Table 23 - Biomass, Algae/Bacteria Ratios, Total N and COD Removal Efficiencies

Reactors / Days	Biomass concentration, mg/l				Algae/bacteria ratio				Total N removal efficiency, percent				COD removal efficiency, percent			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
0	4700	4700	4700		1.8	1.8	1.8									
2	5100	4800	5000		2.9	2.0	3.2		37	42	45		98	88	95	
4	6800	5000	4200		1.6	2.1	2.0		47	44	45		93	93	93	
6	5400	4600	3600		3.5	1.9	2.6		46	55	55		93	90	93	
8	4200	3600	3600		2.5	2.0	2.0		45	49	54		90	93	91	
10	4000	3800	4000		3.0	2.2	1.9		45	53	54		88	88	91	

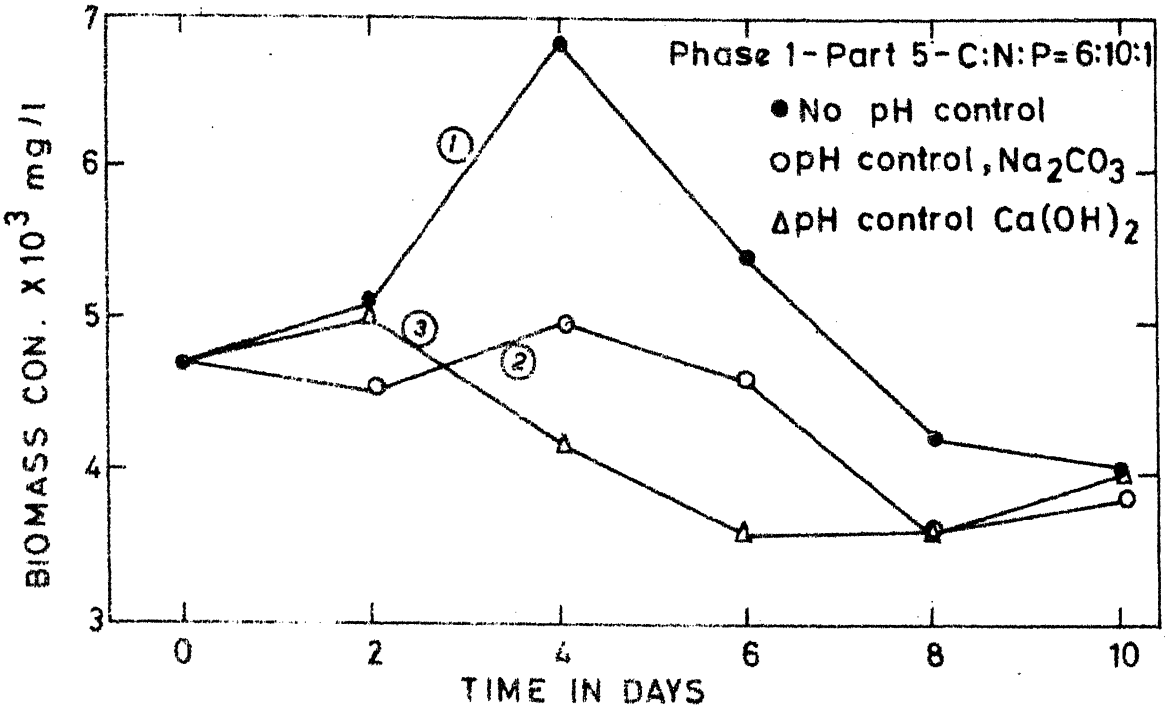


FIG. 29 BIOMASS GROWTH VS. TIME

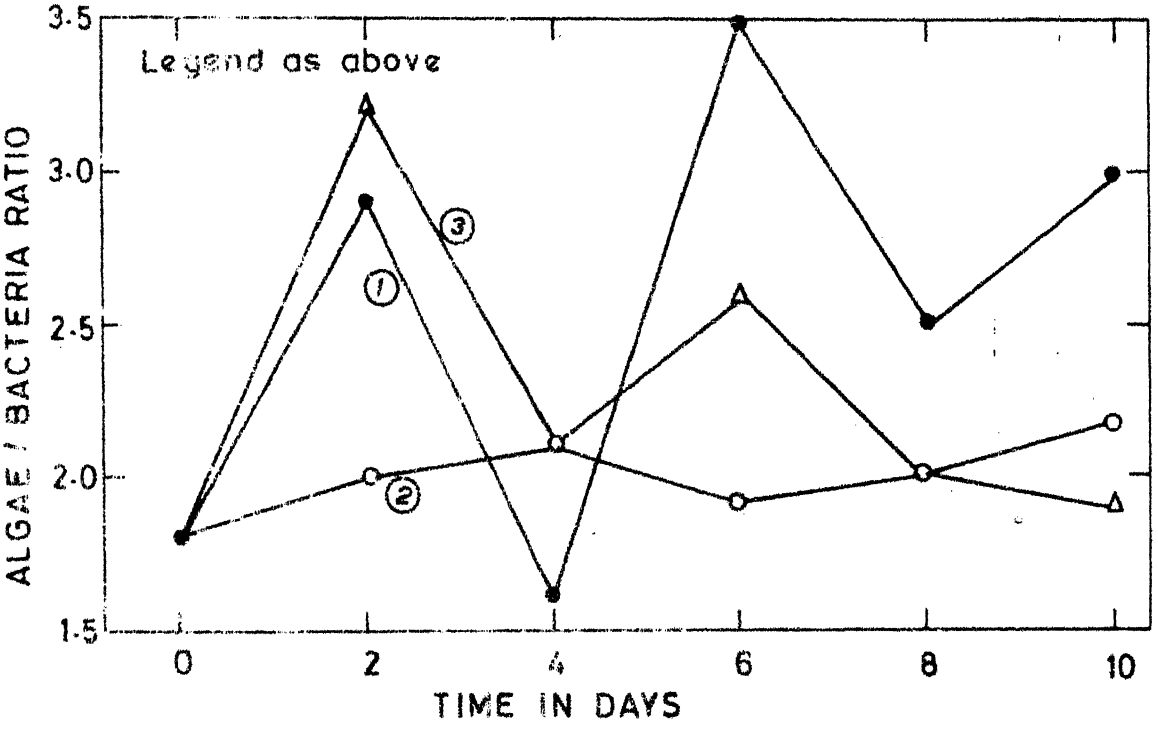


FIG. 30 ALGAE / BACTERIA RATIO VS. TIME

Table 24 - Algal and Bacterial Biomass Concentrations

Days	Reactor 1		Reactor 2		Reactor 3	
	Algae mg/l	Bacteria mg/l	Algae mg/l	Bacteria mg/l	Algae mg/l	Bacteria mg/l
0	3000	1700	3000	1700	3000	1700
2	3800	1300	3000	1500	3800	1200
4	4200	2600	3400	1600	2800	1400
6	4200	1200	3000	1600	2600	1000
8	3000	1200	2400	1200	2400	1200
10	3000	1000	2600	1200	2600	1400

From Table 24 it can be seen that the bacterial biomass was decreasing from 1700 mg/l to 1200 mg/l in Reactor 2 and from 1700 mg/l to 1400 mg/l in Reactor 3. The bacterial biomass concentration in Reactor 1 had decreased from an initial of 1700 mg/l to 1000 mg/l on the 10th day. This indicates that, so far as organic carbon was available, bacterial biomass on the increase and thereafter decreased considerably. The pattern of bacterial biomass in all the reactors was more or less same. But algal biomass concentration was more in Reactor 1 than that in Reactors 2 and 3.

The algae/bacteria ratios were 1.8-3.5 in Reactor 1, 1.8-2.2 in Reactor 2 and 1.8-3.2 in Reactor 3. Algal biomass concentrations in Reactors 2 and 3 were lesser than that in Reactor 1.

The reason for this phenomenon could be attributed to ammonia (NH_3) penetration into cells at pH 8.0-8.3 in Reactors 2 and 3. At this pH inorganic carbon penetration might have been negligibly small and hence further multiplication might not have occurred. However, the pH being 6.5 in Reactor 1, inorganic carbon assimilation might have been rather rapid and hence higher biomass concentration.

The pattern of total N removal efficiency is shown in Figure 31. The efficiency was 37-47 percent in Reactor 1. The corresponding values were 42-58 percent and 45-55 percent respectively in Reactors 2 and 3. The total N removal efficiencies were more at pH 8.0-8.3 in spite of lower biomass concentrations in Reactors 2 and 3. There might have been losses of ammonia due to air-stripping. This works out to be of the order of 10-20 mg/l as indicated in the calculations in Appendix D.

Nitrite N and nitrate N concentrations together ranged from 38 mg/l in Reactor 1 and 54 mg/l in Reactors 2 and 3. The reason for this difference could be the availability of bicarbonate alkalinity and favourable pH for nitrifiers in Reactors 2 and 3 (Adams et.al., 1977; Haug et.al., 1972). Such a condition did not exist in Reactor 1. At pH 8.0-8.3 the flow of inorganic carbon being much less into the algal cells in Reactors 2 and 3, nitrifiers might have obtained considerable amount of inorganic carbon. Hence the presence of higher concentrations of oxidised N in Reactors 2 and 3.

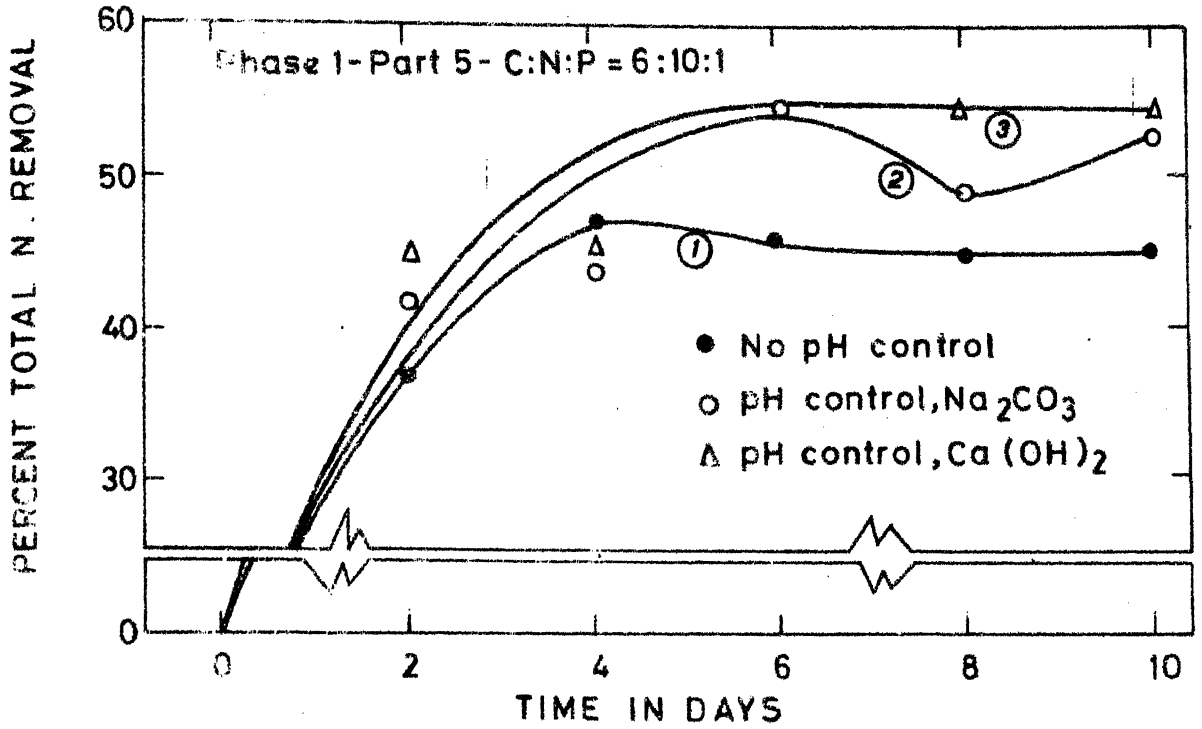


FIG.31 TOTAL NITROGEN REMOVAL VS. TIME

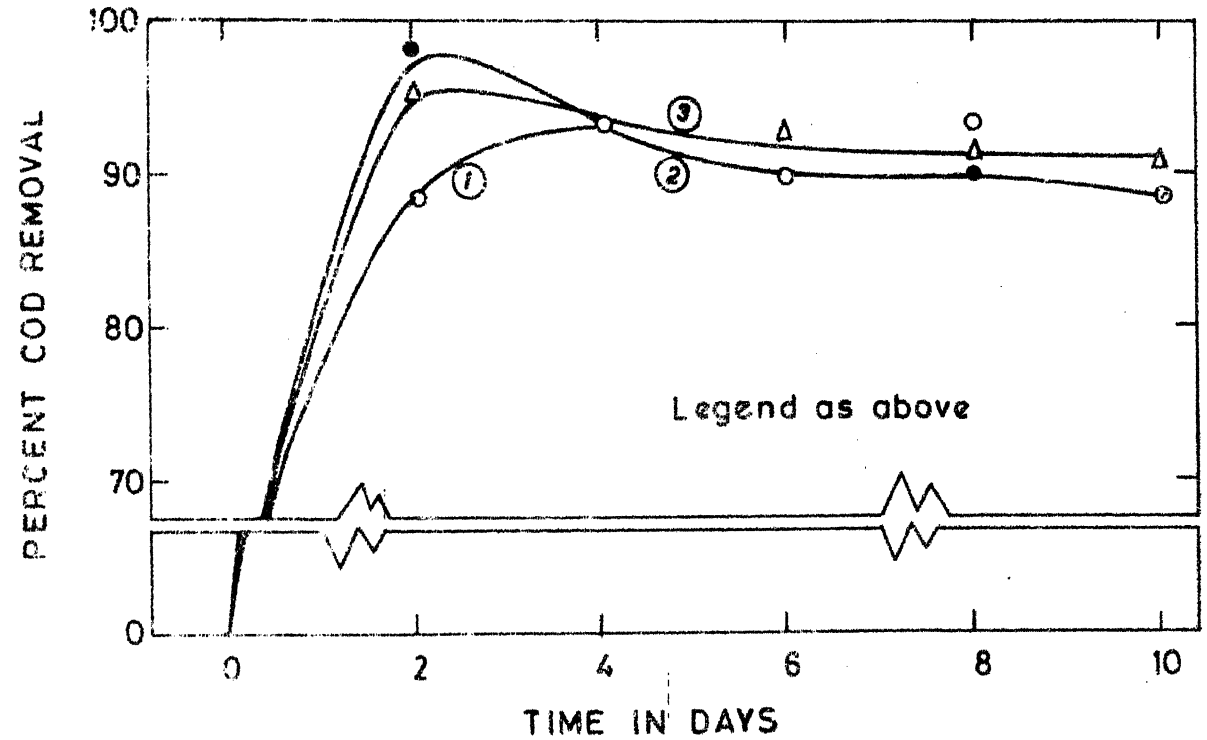


FIG.32 COD REMOVAL VS. TIME

The pattern of COD removal efficiency is shown in Figure 32. The COD removal efficiencies were 95 and 98 percent in Reactors 2 and 3 respectively on 2nd day. The corresponding value was 88 percent in Reactor 1. The reason for this difference is not known. However, the COD removal efficiencies were about 93 percent in all reactors on the 4th and 6th days. This declined to 88-91 percent on the 10th day. This can be attributed to the lysis of biomass as can be seen from Table 24.

The settling values and effluent biomass concentrations are given in Table 25.

Table 25 - Settling Values and Effluent Biomass Concentrations

Reactors / Days	Settling values ml			Effluent biomass concentration, mg/l		
	1	2	3	1	2	3
0	140	140	140	120	120	120
2	140	120	100	100	60	40
6	100	100	100	200	60	40
10	100	80	80	200	50	40

Effluent clarity was better in Reactors 2 and 3 as compared to that in Reactor 1. This is evident from Table 25. With increasing detention times, biomass in the effluent was increasing in Reactor 1. However, the effluent qualities were

improving gradually in Reactors 2 and 3, because settling characteristics of the biomass in those reactors were improving. It is clear from this experiment that a pH of 8.0-8.3 and shorter detention times would be ideal for efficient nitrogen removal. Longer detention times have adverse effects on the performance of Reactors.

6.2.6. Phase 1 - Part 6: Ammonia Removal Study with Activated Sludge System, Algal System, Flocculating Algal-Bacterial System and Simple Aeration:

The procedure is described in Section 5.3.6. The operating conditions are summarised in Table 26.

Table 26 - Operating Conditions in Phase 1 - Part 6 Study, Temperature 5-18°C

Reactor	Biomass used	Ammonia N dose, mg/l	Organic carbon-COD mg/l
1	Activated sludge	1000	1505
2	Algae	1050	1570
3	Algal-bacterial	975	1570
4	No biomass	975	1440

The results obtained in the experiment after acclimatisation are presented in Table 27.

Table 27 - Biomass Concentrations, Total N and COD Removal Efficiencies

Days	Biomass concentration, mg/l			Total N removal efficiency, %				COD removal efficiency %			
	1	2	3	1	2	3	4	1	2	3	4
0	5000	3500	4200								
1	8700	3200	4400	29	33	33		85	66	100	
2	5200	3400	4800	10	31	28	5	75	88	88	5
4	5000	2600	5000	43	48	48	16	72	90	90	76
6	4000	2500	4000	44	51	53	26	87	90	90	89
8	4000	2400	4400	37	51	58	36	95	100	100	100
10	4000	2400	4600	37	51	61	36	100	100	100	

The pattern of biomass increase is illustrated in Figure 33. The activated sludge biomass increased from an initial biomass concentration of 5000 mg/l to 8700 mg/l in one day. However, there was a rapid reduction in biomass from 2nd day onwards. The high increase in biomass might be due to immediate use of organic carbon. Once organic carbon was depleted, the biomass might have entered into a phase of endogenous respiration. This was reflected by biomass reduction. COD curve and drop in biomass indicate that the biomass reduction might have been due to cell lysis. The biomass dropped from an initial concentration of 3500 mg/l to 2400 mg/l on the 10th day in Reactor 2. The normal algal concentration, that could be sustained seems to be 2400-2600 mg/l at the environmental conditions existing.

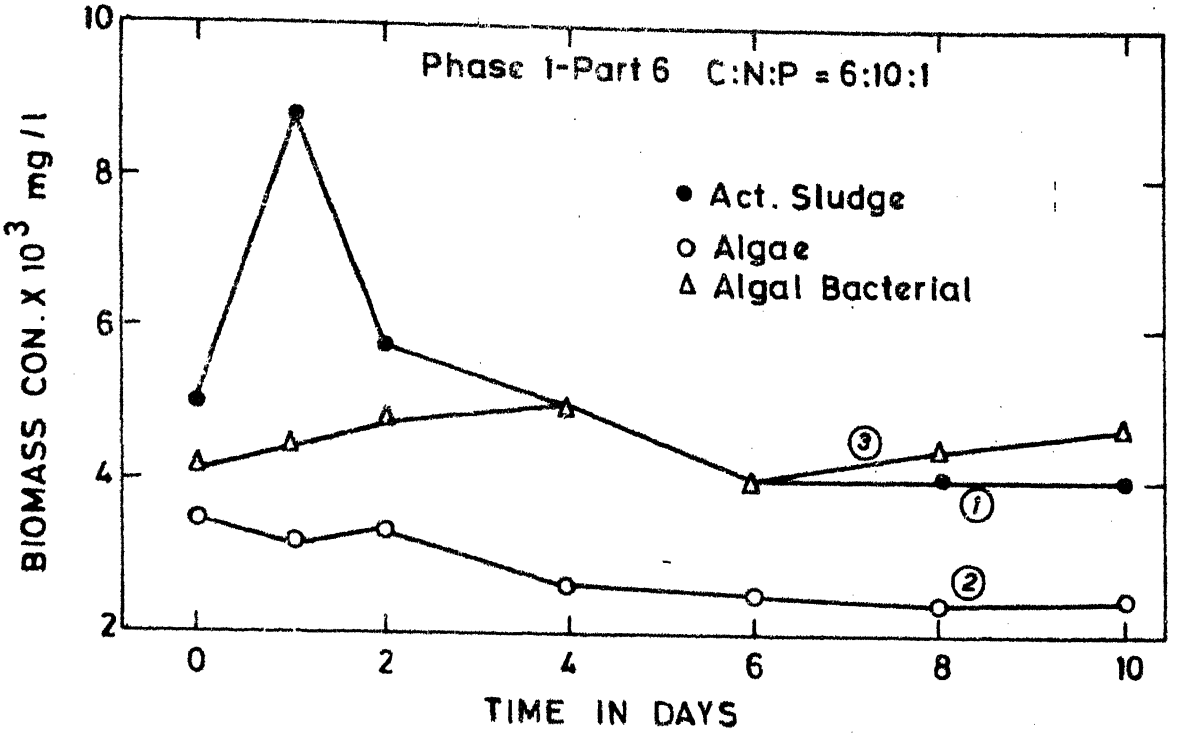


FIG. 33 BIOMASS GROWTH VS. TIME

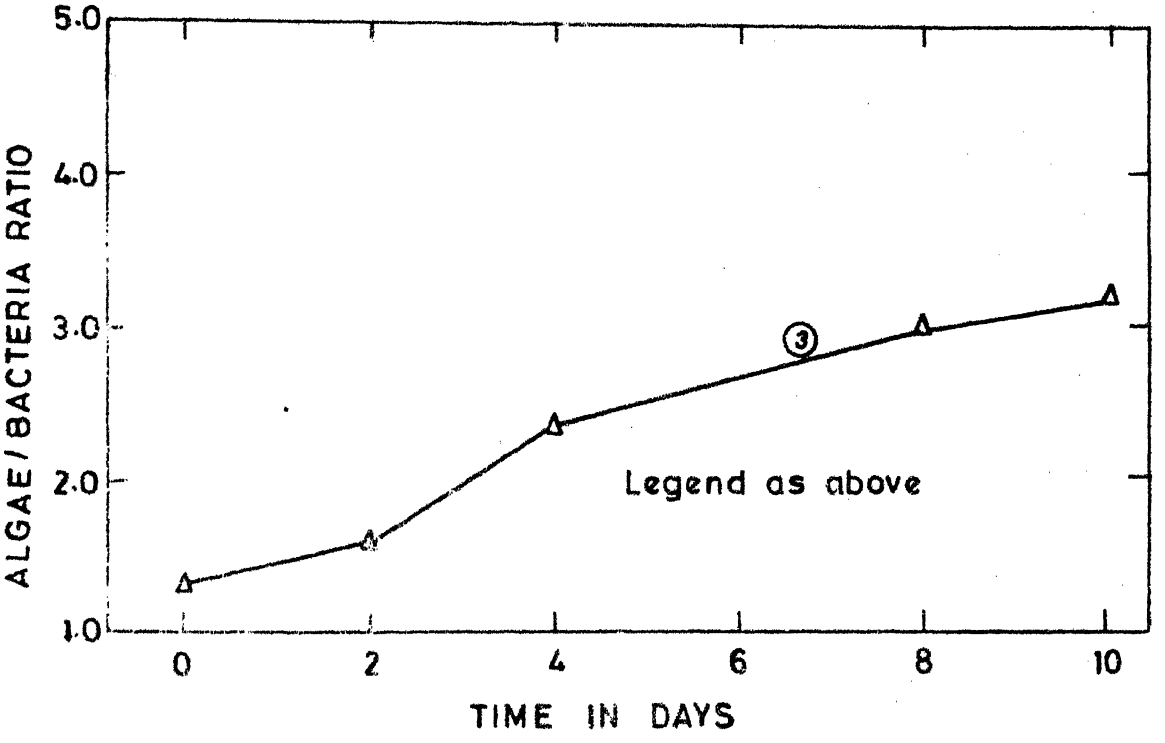


FIG. 34 ALGAE / BACTERIA RATIO VS. TIME

The biomass was not settling at all. The biomass was fluctuating between 4000-5000 mg/l in Reactor 3. There was a gradual increase from an initial biomass concentration of 4400-8000 mg/l on the 4th day. Algae/bacteria ratio is indicated in Figure 34. The feed solution was simply aerated in Reactor 4. No biomass was introduced. It was initially a clear solution. Afterwards it became turbid, probably due to bacterial growth. On the 8th day the whole solution turned green due to algal contamination.

Total N removal efficiency pattern is illustrated in Figure 35. The total N removal efficiency was 43 percent in about 4 days in Reactor 1. After that the efficiency dropped to 37 percent during the next 6 days. There was an increase in NH_3-N, NO_2^--N and NO_3^--N concentrations. Increase in NH_3-N might be due to breakdown of dead cells and endogenous respiration. The efficiency steadily increased from 33 percent on the 1st day to 51 percent on the 10th day in Reactor 2. In spite of gradual biomass reduction, steady increase in total N removal efficiency was a notable feature. This result indicates that it is algae that does the maximum in the nitrogen removal in the system. Nitrites and nitrates were present, but in small quantity. The efficiency was highest in Reactor 3. It increase from 33 percent on the 1st day to 61 percent on the 10th day. COD removal efficiency was 100 percent. Biomass concentration was not decreasing. That might be due to viability of cells and continued ammonia removal. Flocculating

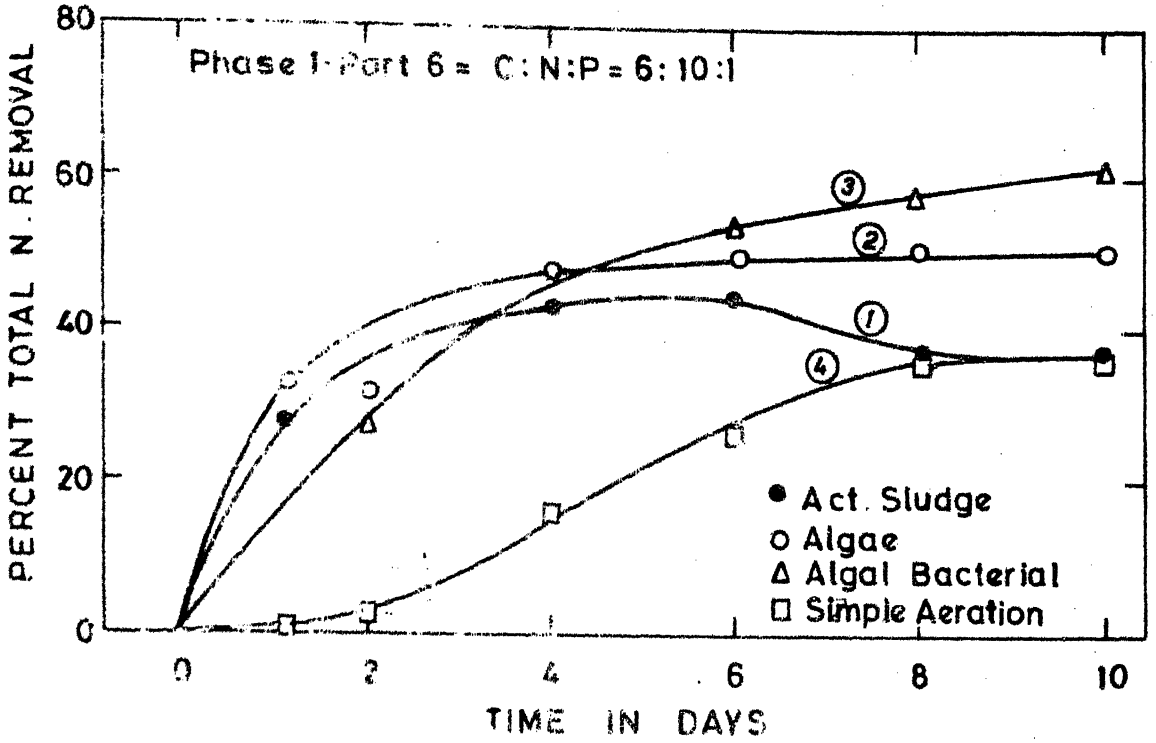


FIG. 35 TOTAL NITROGEN REMOVAL VS. TIME

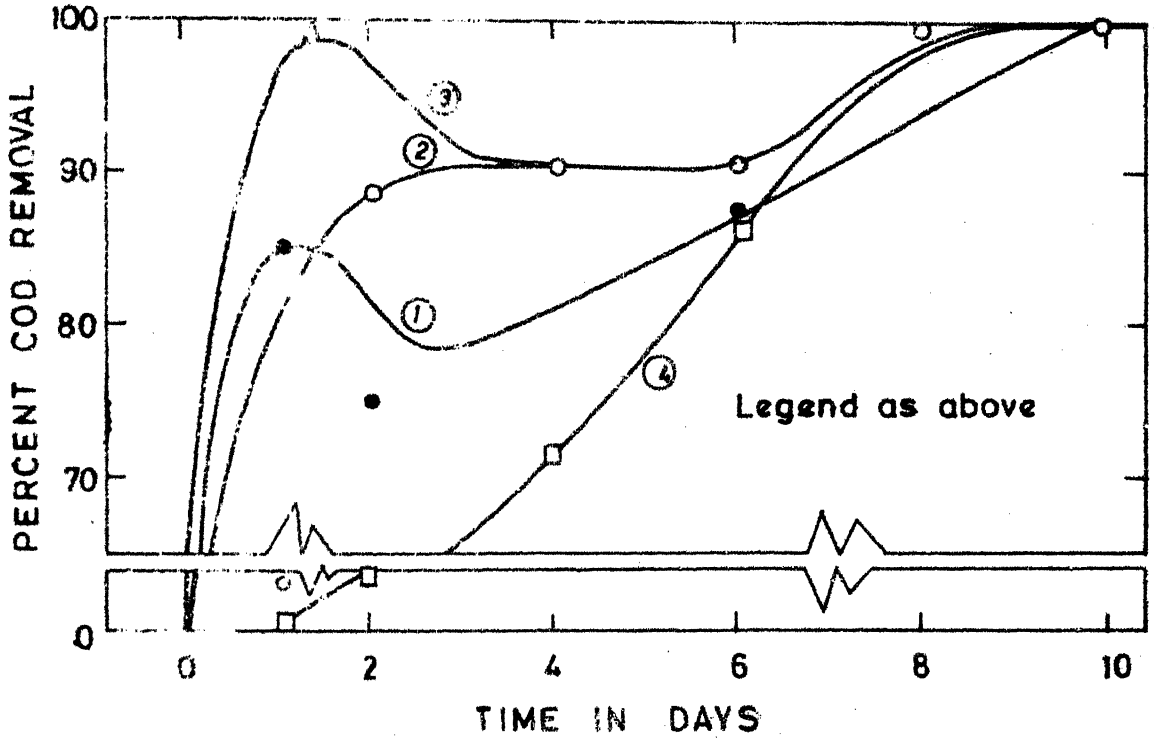


FIG. 36 COD REMOVAL VS. TIME

algal-bacterial system incorporates the advantages of algal system in quick and high rate N removal efficiency and the flocculating property of activated sludge system with additional N removal efficiency. There was no N removal on the 1st day in Reactor 4. However, 5 percent N removal was observed on the 2nd day. Concomitantly there was turbidity in the solution, which might be due to bacterial flora. The removal might have been due to air-stripping also as the pH was 8.0-8.1 during that time. The efficiency steadily increased up to 36 percent on the 10th day.

Nitrite N and nitrate N concentrations were 30-48 mg/l in all reactors. Much difference was not noticeable in Reactors 1, 2 and 3. The concentration was 25 mg/l in Reactor 4. However, nitrification was not predominant in all reactors due to reasons explained in previous sections.

The pattern of COD removal efficiency is shown in Figure 36. COD removal efficiency was 85 percent on the 1st day and dropped down to 75 percent on the 2nd day in Reactor 1. Thereafter it was gradually increasing to 100 percent on the 10th day. The rise in COD removal efficiency during the later part might be due to the utilisation of organic materials from lysed cells by living cells. The efficiency was rapid for 2 days in Reactor 2. Thereafter the rise was gradual.

pH dropped to 7.0 from an initial value of 8.0 in all reactors. The alkalinity dropped from an initial concentration of 470 mg/l to 70 mg/l during 10 days in all reactors.

Settling values of activated sludge was 50 ml on the 10th day. It was 120 ml initially. This is indicated in Table 28.

Table 28 - Settling Values

Days	Settling values in ml	
	1	3
0	120	130
4	80	120
8	60	80
10	50	60

The settling value was 130 ml initially, but dropped to 60 ml on the 10th day in Reactor 3. The effluent biomass concentration was 50 mg/l on the 2nd day, but rose to 120 mg/l on the 10th day in Reactor 3.

Algae/bacteria ratio in Reactor 3 was 1.33-1.5. However, the value of 2.3 on the 4th day and 3.2 on the 10th day. The same trend was observed in earlier experiments. The biomass in Reactor 2 was not at all settling.

These experiments indicated that flocculating algal-bacterial system is superior to activated sludge system with respect to N removal and superior to algal system with respect to auto-flocculation and N removal.

6.2.7. Phase 1 - Part 7: Urea and Ammonia Removal Study with C:N:P Ratios of 5:10:1, 7:10:1, 9:10:1 and 10:10:1:

Urea was added besides ammonia in this study. The approximate proportion of ammonia N to urea N was 7:5, the total quantity being fixed at about 1000 mg/l N. The biomass was acclimatised to this feed as explained in Section 5.3.7. From earlier studies it was found that the C:N:P ratio can be advantageously lowered down to 6:10:1. However, higher values were used because urea degradation require more of bacteria, which in turn require additional quantity of organic carbon. The operating conditions in Phase 1 - Part 7 are summarised in Table 29.

Table 29 - Operating Conditions in Phase 1 - Part 7 Study, Temperature 15-28°C

Reactors	C:N:P ratio	Ammonia N dose, mg/l	Urea N dose mg/l	Organic carbon-COD, mg/l
1	5:10:1	650	290	1270
2	7:10:1	650	310	1800
3	9:10:1	675	310	2300
4	10:10:1	675	300	2690

Table 30 illustrates the patterns of biomass growth, algae/bacteria ratio, total N and COD removal efficiencies.

Table 30 - Biomass, Algae/Bacteria Ratios, Total N and COD Removal Efficiencies

Reactors / Days	Biomass concentration, mg/l				Algae/bacteria ratio				Total N removal efficiency, percent				COD removal efficiency, percent			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
0	4000	4000	4200	4500	1.3	1.3	1.6	1.3								
2	5400	5600	5600	5800	1.1	1.0	1.1	1.2	52	51	59	56	58	83	97	100
4	4800	4000	4800	5000	1.1	1.7	1.3	1.5	46	51	57	62	74	83	82	74
6	3800	3800	3500	3900	2.2	1.9	2.5	3.3	44	56	57	55	60	83	73	80
8	3300	3400	4000	4800	5.6	5.8	4.0	2.4	51	51	51	56	51	84	78	85
10	3500	3100	3700	4000	4.8	5.2	5.1	4.0	45	51	43	52	45	62	70	78

The pattern of biomass growth can be seen from Figure 37. The growth pattern was generally similar in all reactors. The biomass concentration went up to 5400, 5600, 5600 and 5800 mg/l in Reactors 1, 2, 3 and 4 respectively on the 2nd day. Thereafter the biomass concentrations declined in all reactors. However, there was an increase in biomass concentrations from the 6th day onwards in Reactors 3 and 4. This was due to gradual increase in algal biomass as evident from Figure 38.

Algae/bacteria ratios were fluctuating between 1.1-1.7 in all reactors. However, after the 8th day the above values rose up to the order of 2.4-5.8 in all reactors. Where C:N:P ratio was less, algae/bacteria ratio was more and vice versa.

The pattern of total N removal efficiency is shown in Figure 39. It could be seen from the figure that the total N removal efficiency gradually dropped in Reactor 1 after 2nd day. However, the fall in efficiency in other reactors began from 4th day onwards. This might be possibly due to the presence of organic nitrogen in the effluent. There was not much of organic N in the effluent up to the 4th day. The increase in the effluent ammonia N could also be due to the release of absorbed ammonia from the cell. This is justified from sludge N content at various detention times (Appendix C.7). The total N removal efficiencies were 46 percent, 51 percent, 59 percent and 56 percent in Reactors 1, 2, 3 and 4 respectively on the 2nd day. The efficiency rose up to 62 percent on the

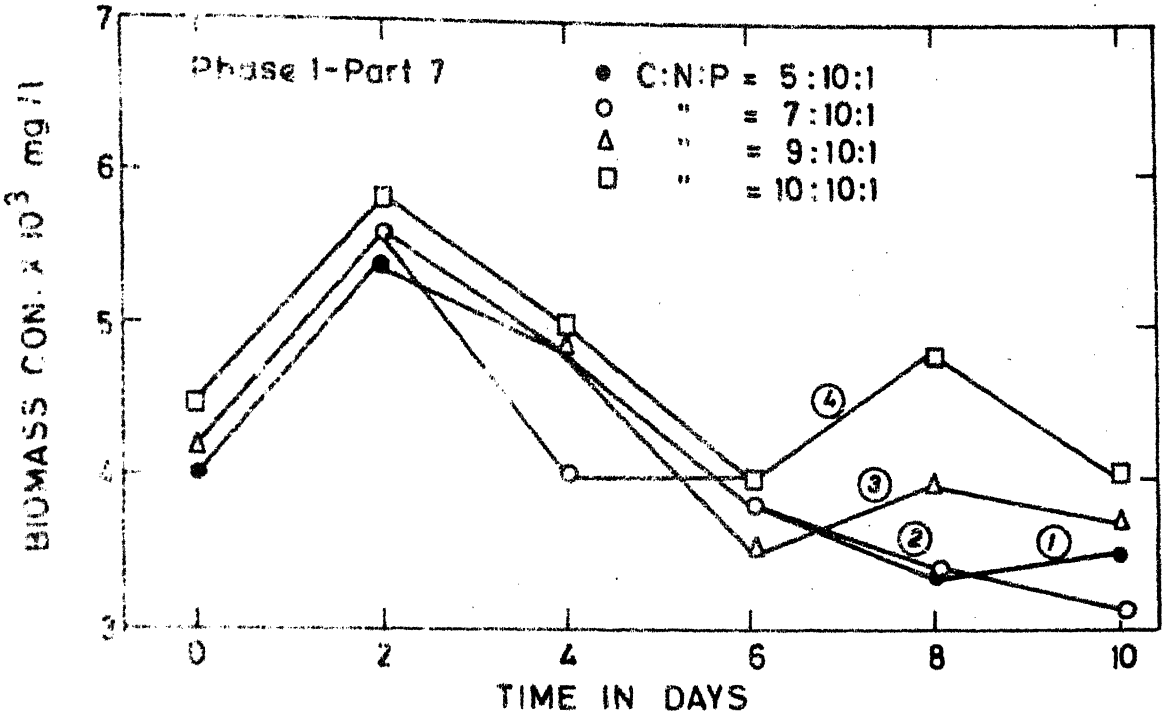


FIG 37 BIOMASS GROWTH VS. TIME

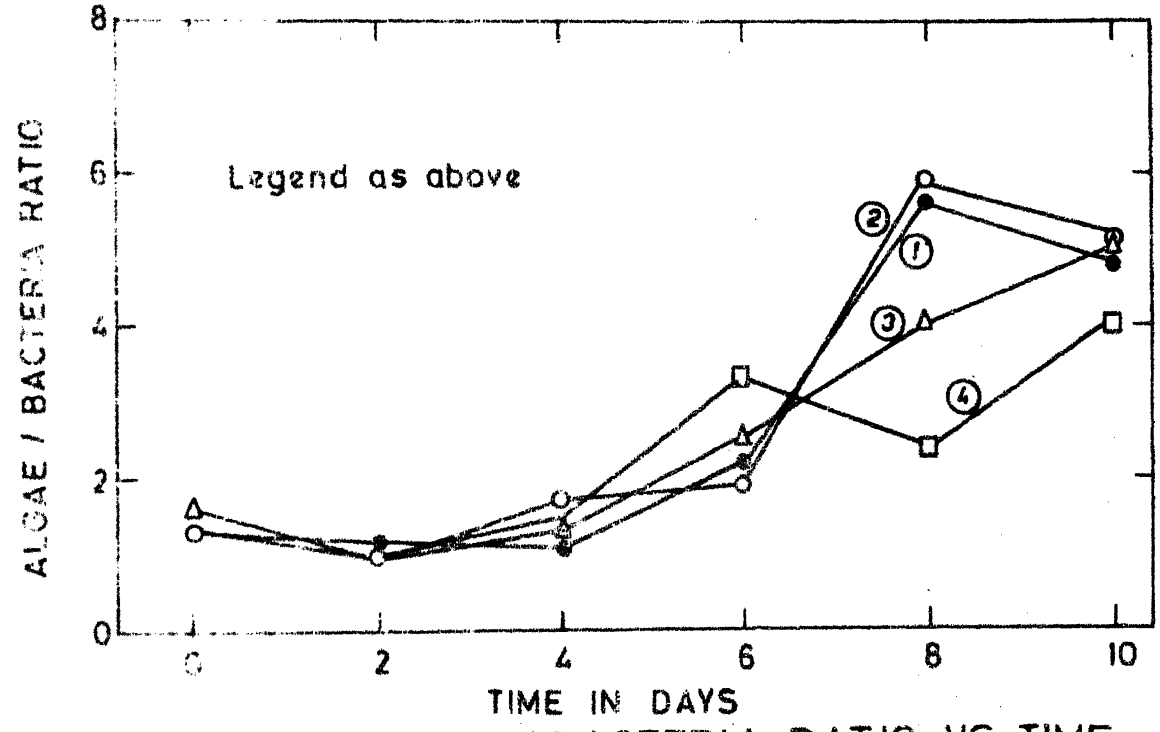


FIG. 38 ALGAE / BACTERIA RATIO VS. TIME

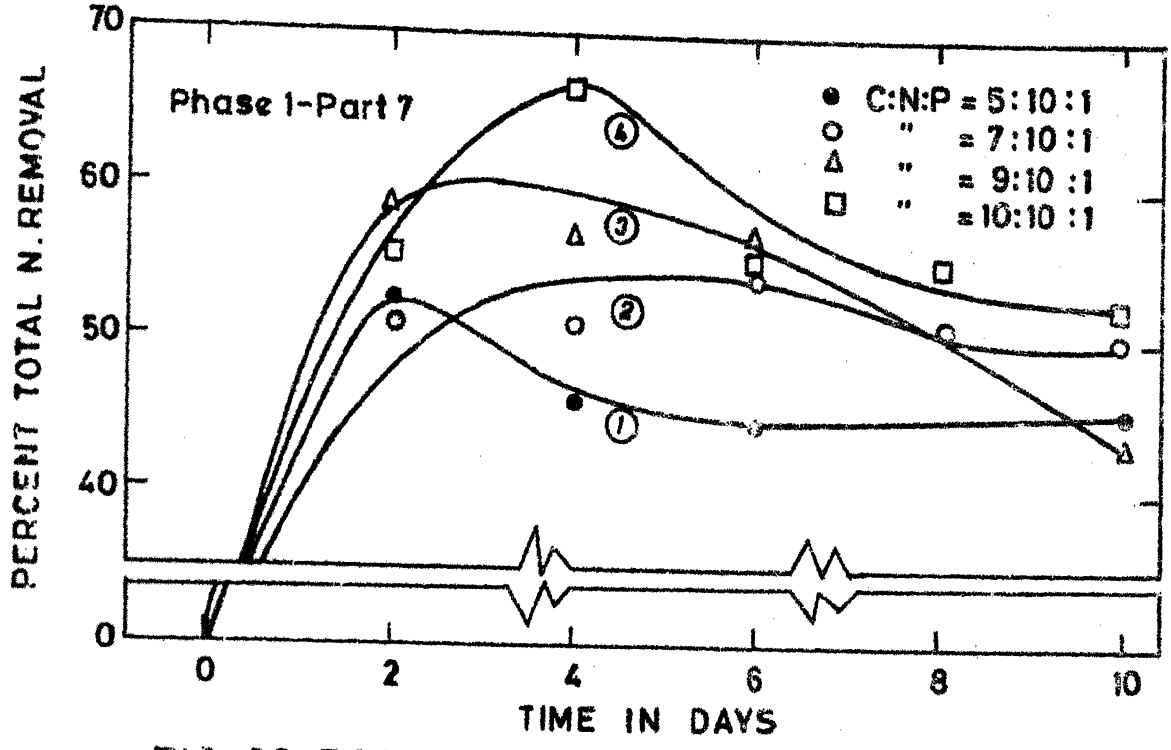


FIG. 39 TOTAL NITROGEN REMOVAL VS. TIME

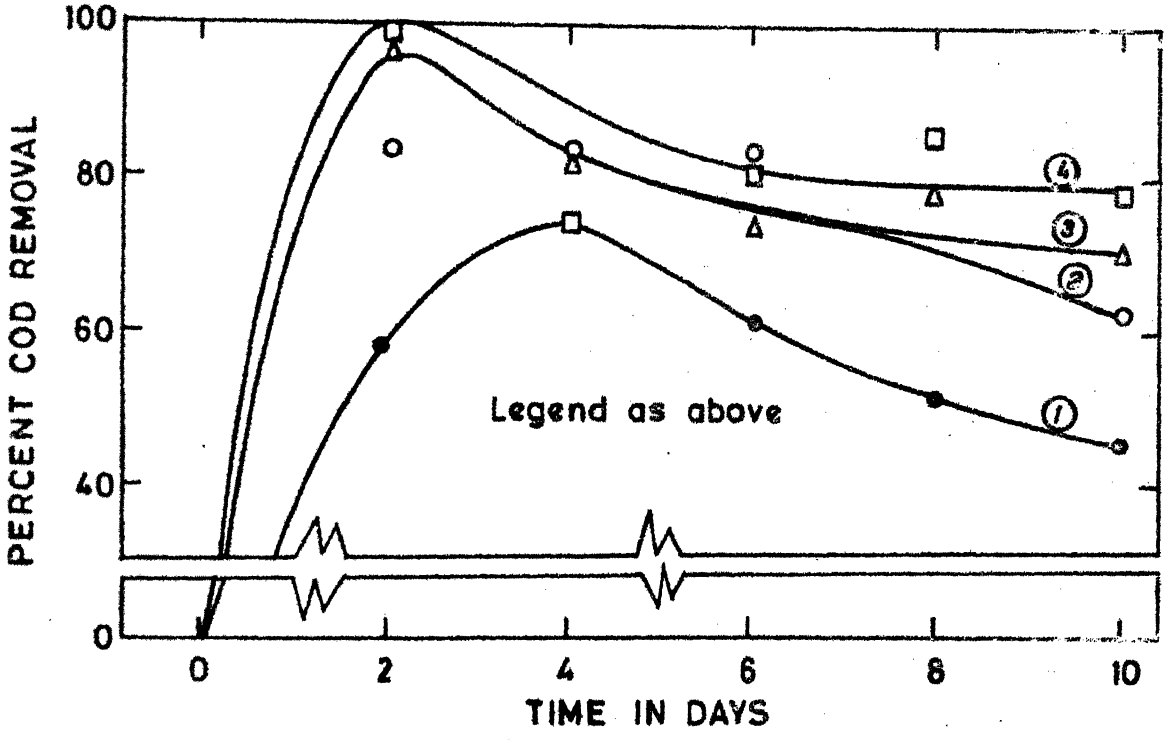


FIG. 40 COD REMOVAL VS. TIME

4th day in Reactor 4. From these facts it is clear that, as the C:N:P ratio was higher, the biomass stability was more and so also the total N removal efficiency. About 4 percent more efficiency was observed in reactors with higher C:N:P ratios against the preceeding ones. Too much of organic carbon would involve more cost and so the ratio beyond 10:10:1 was not tried.

Total absence of urea after the 4th day in Reactor 4 was a notable feature. The urea N concentrations on the 4th day were 40 mg/l, 50 mg/l and 60 mg/l in Reactors 3, 2 and 1 respectively. This revealed that organic carbon had a favourable effect on the conversion of urea N into ammonia N. The presence of ammonia effectively prevents the uptake of urea by algae in an algal culture (McCarthy, 1971; Williams et.al., 1977). According to Hattori (1957) ammonia compared to urea is preferentially taken by algae. So the urea removal in flocculating algal-bacterial system might have been due to its degradation by bacterial and chemical means. Hence the bacterial flora are of immense help in this respect. It is in this perspective that the necessity of organic carbon must be viewed. These facts were borne out by experimental results in this study. The more the organic carbon present, the more was urea removal efficiency.

There was a reduction in bacterial biomass after 4th day (Appendix C.7). Within that period all urea was degraded.

Moreover, it can be observed from the experimental results that a C:N:P ratio of 10:10:1 is required if urea degradation is to be very effective.

Concentrations of nitrites and nitrates together were 90, 72, 82 and 80 mg/l in Reactors 1, 2, 3 and 4 respectively. The pattern of COD removal efficiency is shown in Figure 40. Maximum COD removal efficiency of 83, 93 and 100 percent were obtained in Reactors 2, 3 and 4 respectively on the 2nd day. However, a maximum COD removal efficiency of only 74 percent was obtained in Reactor 1 on the 4th day. Afterwards the efficiency dropped still further. This must be read along with the biomass concentration in Reactor 1. Similar were the patterns in Reactors 2, 3 and 4 after the 2nd day.

It is presumed that the COD in the effluents of all reactors after 2nd or 4th day as the case may be, might be due to organics that might have originated from decayed cells. This also points out that in a batch reactor, COD removal efficiency falls down after 2 days.

6.3. Luxury Uptake of Nitrogen:

Experiments conducted in Phase 1 - Part 7 study revealed the existence of luxury uptake of nitrogen by the algal-bacterial biomass. Table 31 shows the details. It is clear from the table that the total sludge N was increasing up to certain period and thereafter there was a release of ammonia into the effluent.

Table 31 - Biomass Concentration and Biomass Nitrogen Content

Days	C:N:P = 8:10:1			C:N:P = 7:10:1			C:N:P = 9:10:1			C:N:P = 10:10:1		
	Biomass concentration mg/l	Sludge N mg/l	Biomass N content percent	Biomass concentration mg/l	Sludge N mg/l	Biomass N content percent	Biomass concentration mg/l	Sludge N mg/l	Biomass N content percent	Biomass concentration mg/l	Sludge N mg/l	Biomass N content percent
2	5400	500	9.30	5600	500	9.10	5600	500	9.10	5800	550	9.48
4	4800	550	11.46	4000	550	13.75	4800	525	11.00	5000	600	12.00
6	3800	550	14.47	3800	600	16.00	3500	550	15.71	3900	525	13.46
8	3300	500	15.15	3400	500	15.10	4000	500	12.50	4800	500	10.42
10	3500	500	14.28	3100	500	14.30	3700	400	11.50	4000	500	12.50

The patterns of biomass variation, sludge N content and pH are shown in Figures 41, 42, 43 and 44 for Reactors 1, 2, 3 and 4 respectively. The changes of biomass, pH and nitrogen content of biomass were following more or less same trend in all reactors. It can be seen from the table that the percent N in the biomass increased rapidly up to 6th day in all reactors.

Percent N content of biomass in Reactor 1 rose from 9.3 on the 2nd day to 14.47 on the 6th day and 15.15 on the 8th day. Though there was biomass reduction after the 2nd day, the total N content of the biomass was increasing up to the 6th day. After that total N content declined. The pH dropped from an initial value of 8.2-to 6.6 on the 6th day. Figure 41 clearly indicates a close relationship between pH, biomass and biomass N content. Increase in total N content in the biomass, in spite of biomass decline, might be due to continued uptake of ammonia by algal cells. There was decline in bacterial biomass (Appendix C.7); and it might be due to this that there was organic N in the effluent after the 4th day. Initially at a pH 8.0 when organic carbon was not very much limiting, ammonia uptake was increasing as indicated by the curve. The maximum sludge N percentage was found to be 15.15 in Reactor 1.

Figure 42 shows the trend for Reactor 2. The curves follow the same pattern as in the case of Reactor 1. Same arguments hold good here too. The maximum sludge N percentage was 16.00

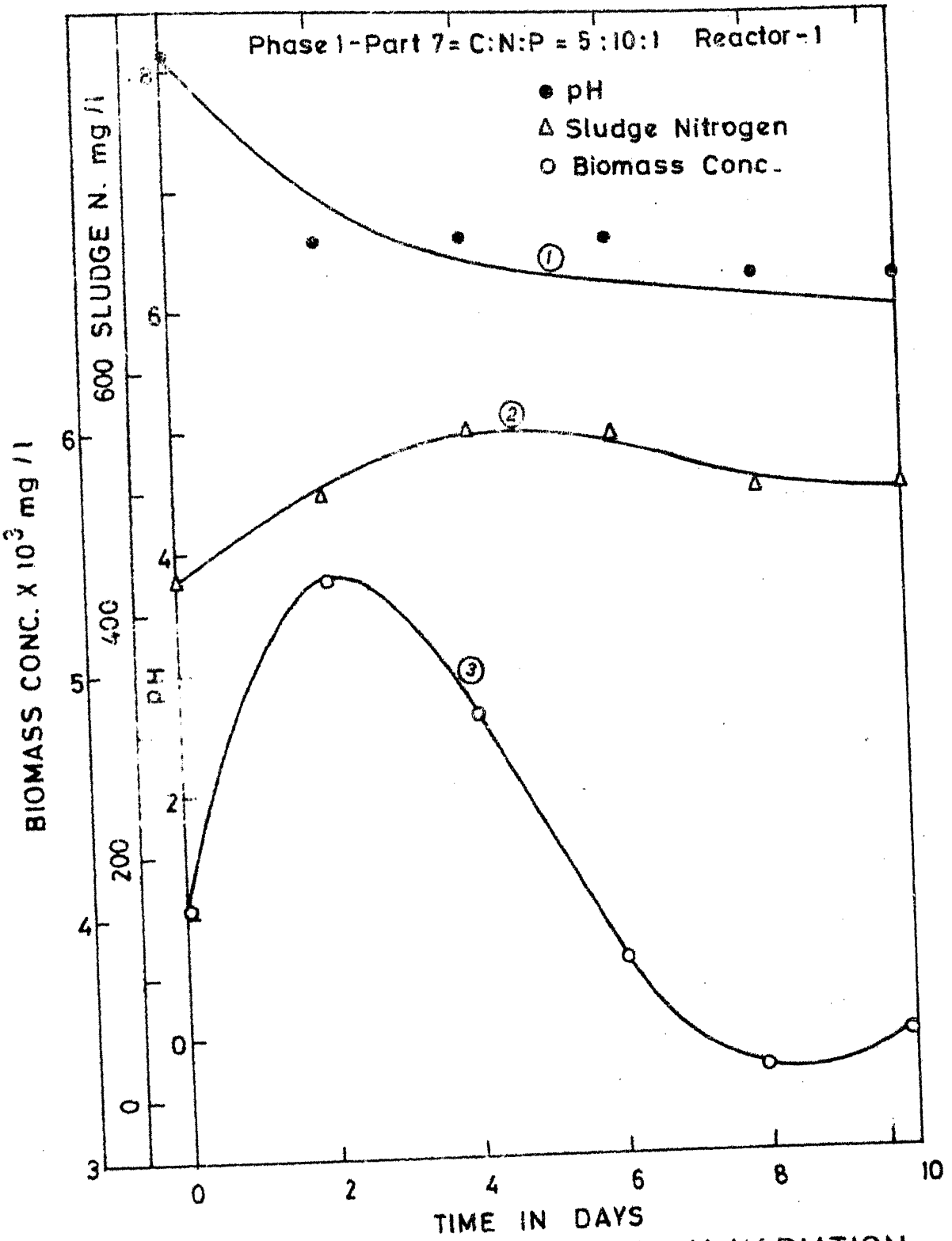


FIG. 41 SLUDGE NITROGEN-BIOMASS-pH VARIATION

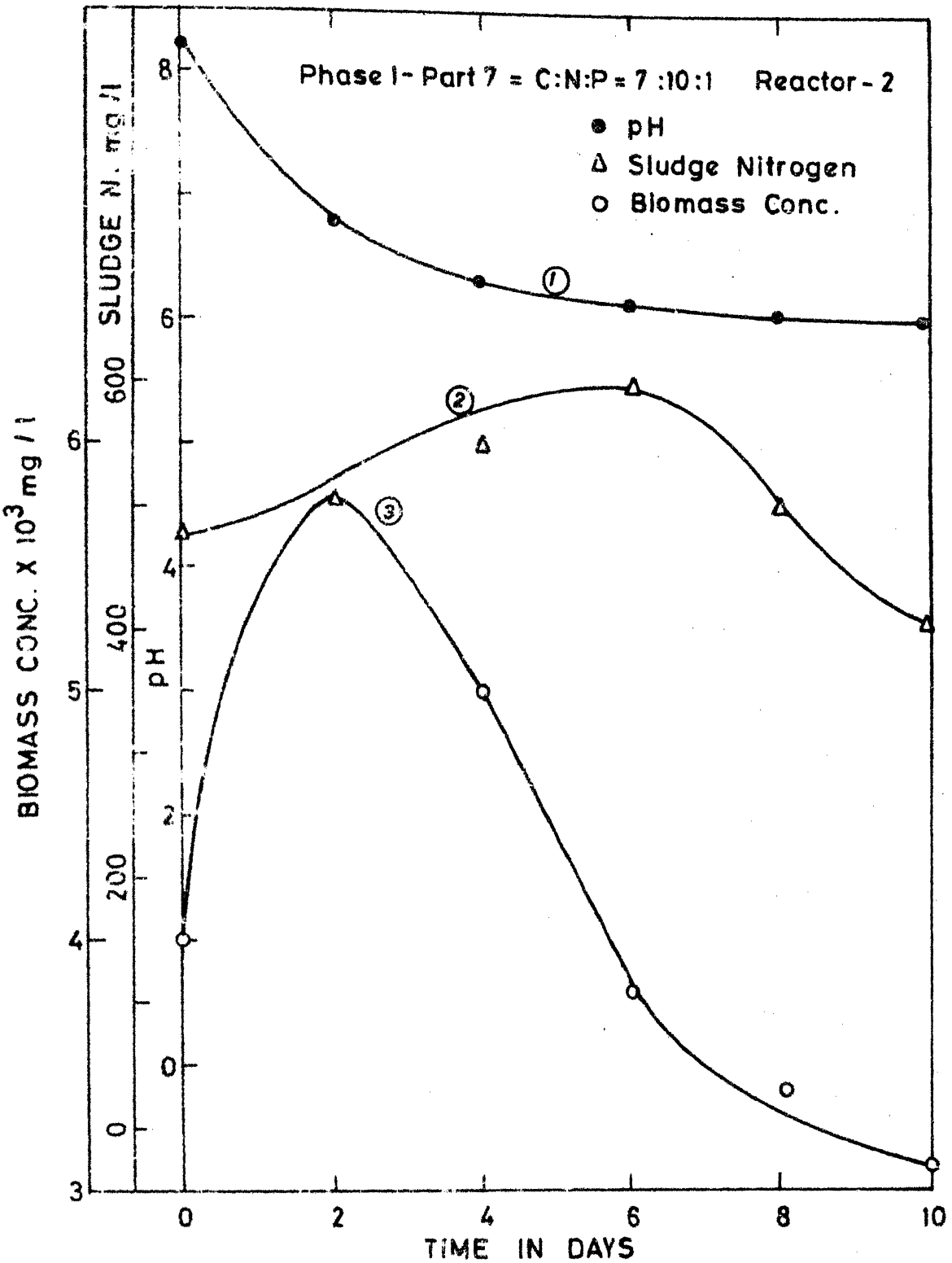


FIG. 42 SLUDGE NITROGEN - BIOMASS - pH VARIATION

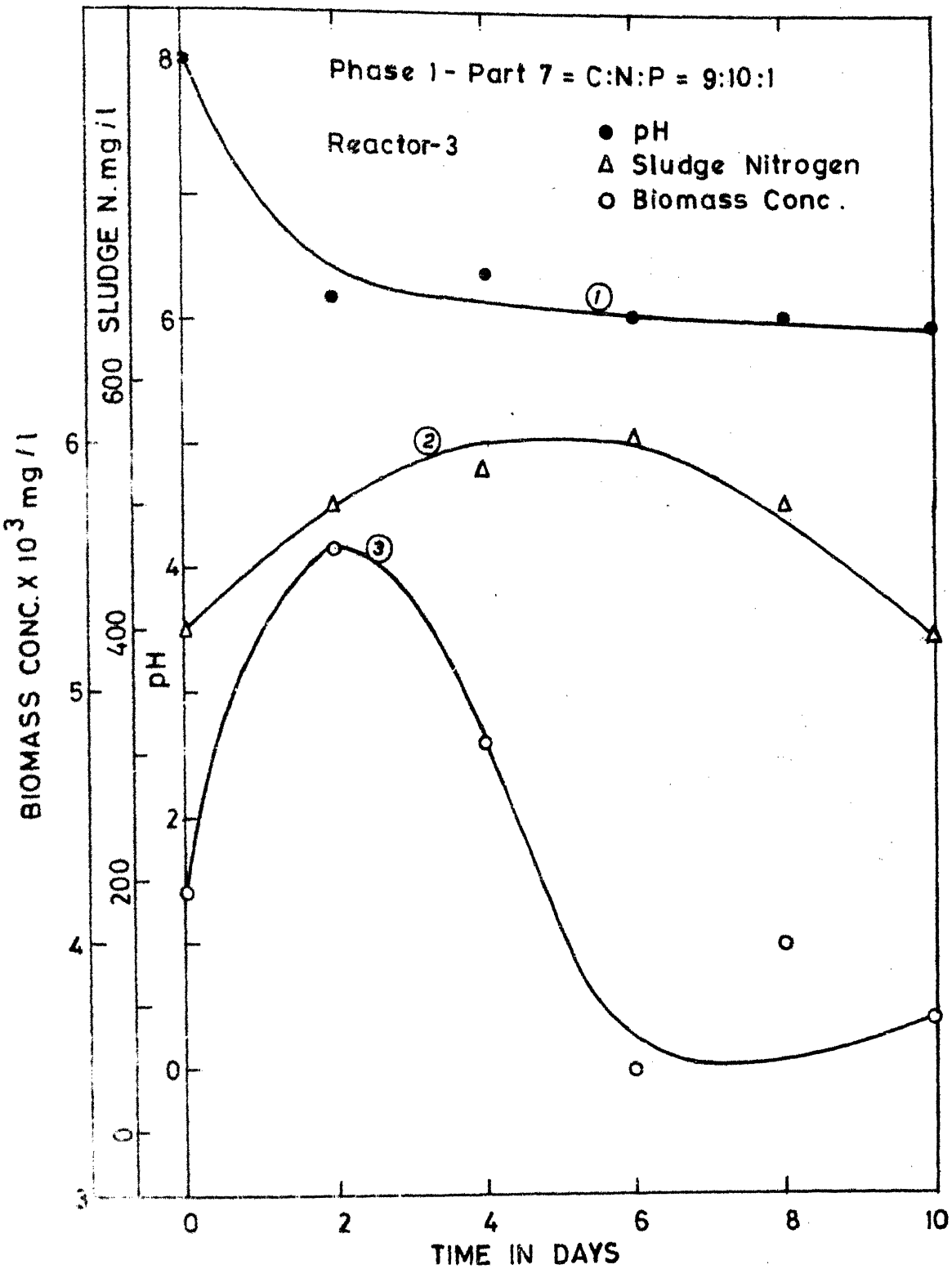


FIG. 43 SLUDGE NITROGEN-BIOMASS-pH VARIATION

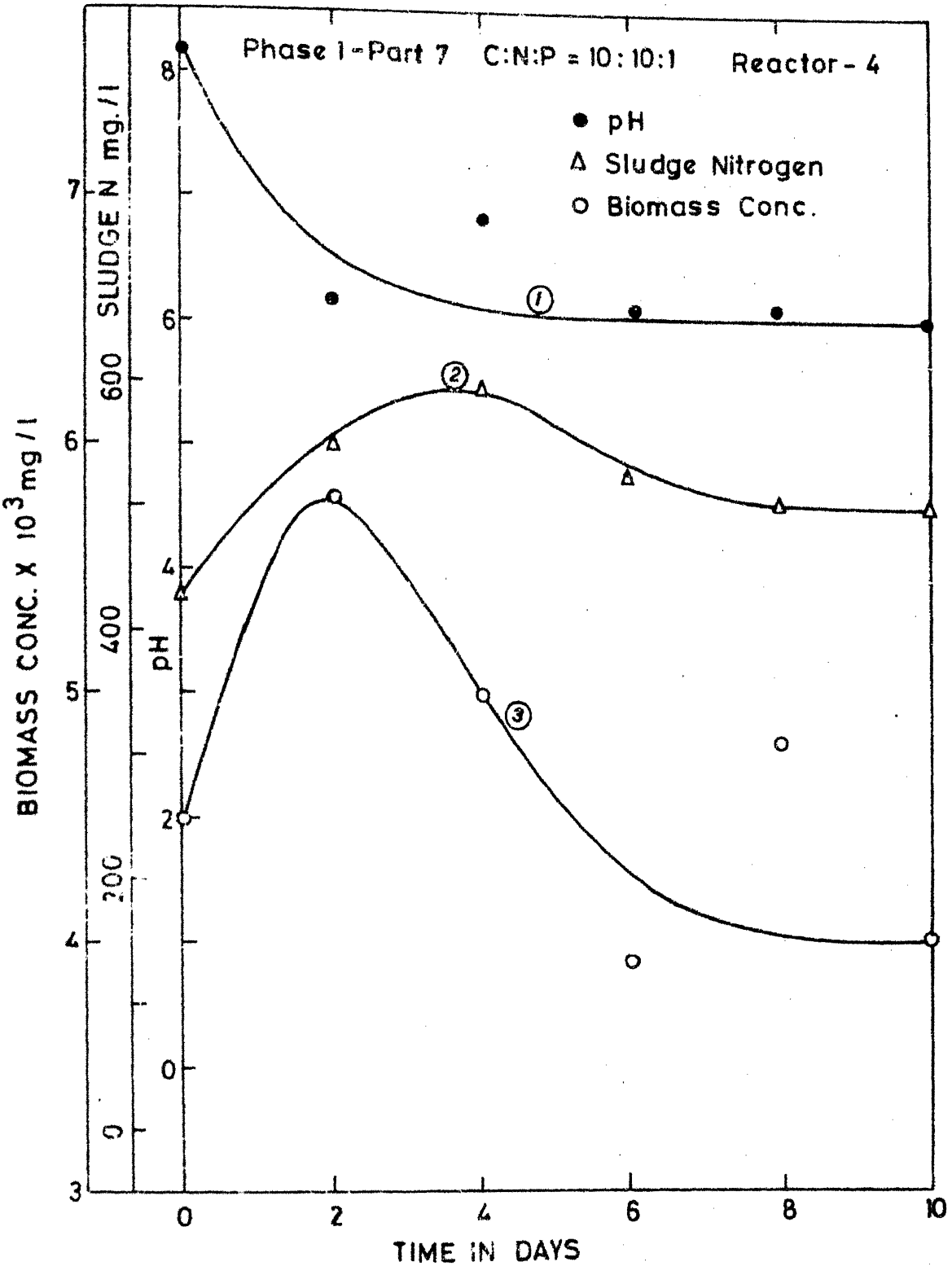


FIG. 44 SLUDGE NITROGEN-BIOMASS-pH VARIATION

in Reactor 2, occurring on the 6th day. The maximum sludge N percent in the biomass in Reactor 3 was 15.71 occurring on the 6th day. The pattern of biomass, pH and biomass N content variation is shown in Figure 43. Same arguments put forth earlier hold good here too. Figure 44 shows the pattern of changes of biomass, pH and total N in the biomass in Reactor 4. The maximum total N percent of biomass in this reactor was 13.46. It was possibly due to the presence of more of bacterial flora.

So it can be inferred that organic carbon might indirectly reduce the N percentage of the biomass. However, it was observed that there was a tendency for the biomass to incorporate more of nitrogen than present in normal cells. From literature it is observed that algae normally contains about 5-10 percent of N by weight of biomass. In this study it was consistently observed that the total N removal and its incorporation into the biomass ranged between 9-16 percent of the biomass. This clearly indicates the existence of luxury uptake of nitrogen by the biomass at pH 8.0-8.3 and hence at carbon stressed conditions.

The incorporation mechanism can be explained as follows. When carbon stress is developed cell division rate is retarded. As a result the cells might become enlarged (Pipes, 1961; Oswald et.al., 1953; Chrost et.al., 1975). Moreover, the enlargement of the algal cells might also be due to the

presence of organic carbon (Zazic, 1970). As a result of cell enlargement and carbon stress at high pH, ammonia (NH_3) can passively flow into the cells (Dohler, 1971). Even at lower pH, ionised ammonia (NH_4^+) might pass, though to a small extent (Warren, 1962). Due to the above effects luxury uptake of nitrogen might have taken place. So it can be inferred that luxury uptake of nitrogen does exist in algal-bacterial cultures exposed to high concentrations of ammonia and urea. This occurs at detention times of 4-6 days in batch reactors.

6.4. Nitrogen Mass Balance:

The nitrogen mass balance was done at the end of 2nd, 4th, 6th, 8th and 10th days for all the four reactors. Complete data are presented in Appendix C.7. A typical nitrogen mass balance chart is shown in Figure 45. From this chart it is clear that bulk of urea was degraded within 2 days. Most of the nitrogen removed, was incorporated in the biomass. Comparatively small proportions were converted into nitrites and nitrates. The loss of ammonia due to air-stripping was calculated according to the formula given by Loehr et.al. (1973). The detailed calculation of quantity lost due to air-stripping is presented in Appendix D. The total ammonia N lost due to air-stripping under the experimental conditions averaged to 20 mg/l in 2 days. It can be seen that the mass balance was more or less complete in the case of Reactors 3 and 4, as unaccounted forms were negligibly small. However,

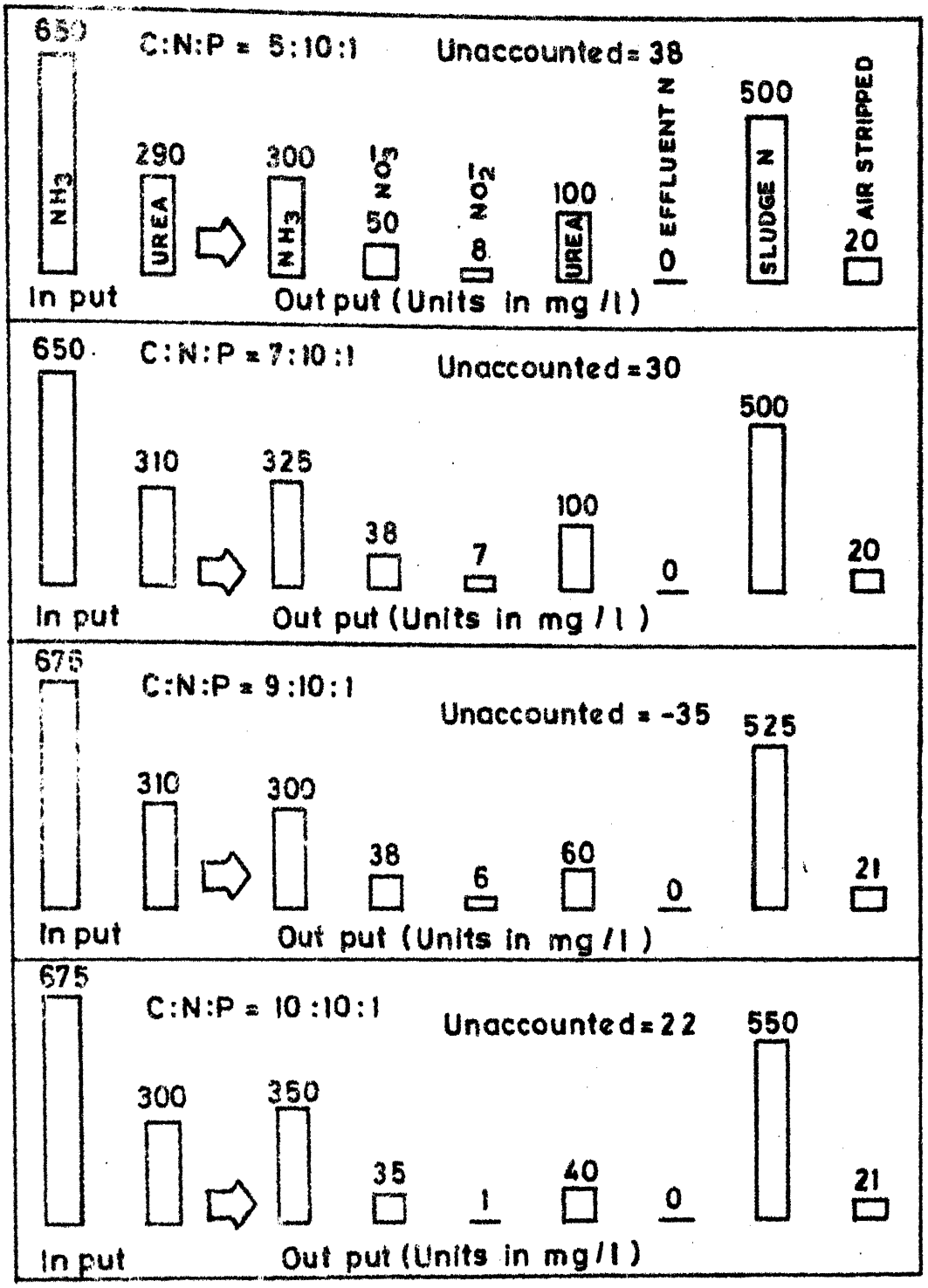


FIG. 45 TYPICAL NITROGEN MASS BALANCE CHART

the balance was not much satisfactory in the case of Reactors 1 and 2. The reason for this could not be accounted for.

6.5. Summary of Phase 1 Studies:

The important features that could be noticed in the batch studies are given below. In this phase total N feed was more or less 1000 mg/l.

1. The higher the C:N:P ratio, the larger the stabilised biomass concentration.
2. The lowest C:N:P ratio for treating ammonia N alone seems to be 6:10:1. However, the total N removal efficiency was 56 percent as against 80 percent when the C:N:P ratio was 30:10:1.
3. The optimum C:N:P ratio for treating ammonia N and urea N seems to be 10:10:1. This was based on the total N removal efficiency, settleability and COD removal efficiency.
4. The higher the C:N:P ratio, the lower the algae/bacteria ratio.
5. Carbon dioxide is apparently not required for removal of ammonia N from high nitrogenous wastes.
6. Since sewage contains small amount of organic carbon, it cannot be used as a source of organic carbon in the treatment of undiluted high nitrogenous wastes.

7. As the detention time increased beyond two days, the total N removal efficiency improved only to a small extent. But the biomass concentration and COD concentration in the effluent were increasing as detention time increased.
8. At a C:N:P ratio of 6:10:1, the maximum biomass concentration attained was 4400 mg/l, whereas at a C:N:P ratio of 10:10:1 the same was 5800 mg/l.
9. The higher the C:N:P ratio, the better were the flocculating and settling properties of the biomass.
10. The flocculating algal-bacterial system seems to be the best among other biological systems such as activated sludge and algal system in terms of total N removal efficiency and biomass settling characteristics.
11. Total N removal efficiency is considerably enhanced when pH is maintained in the range of 8.0-8.3.
12. The shorter the detention time, the lesser the effluent biomass concentration and organic N concentration.
13. The longer the detention time (up to 6 days) the greater the amount of nitrogen incorporated in the biomass and hence the more efficient the total N removal.
14. Luxury uptake of nitrogen takes place in the flocculating algal-bacterial system in the pH ranges of 8.0-8.3.
15. The optimum operating parameters in batch study are: detention time - 2 days; C:N:P ratio - 10:10:1; pH - 8.0-8.3; Aeration - 1 lpm/l; biomass concentration - 5000-6000 mg/l.

6.6. Phase 2: Ammonia and Urea Removal Study with Batch Reactors in Series:

Detailed experimental procedure is given in Section 5.4. 3 batch reactors were operated in series in order to obtain successive removal of total N. The feed was having a C:N:P ratio of 10:10:1 with ammonia N concentration of 700 mg/l and urea N concentration of 300 mg/l. A detention time of 2 days was adopted for each reactor, as total N removal efficiency was appreciably high, as revealed by batch experiments. Moreover, at a detention time of 2 days, effluent contained little of organic N. A biomass concentration of 4800-5400 mg/l was chosen as it was the concentration that could be developed and sustained by a feed containing about 1000 mg/l N with a C:N:P ratio of 10:10:1. At this C:N:P ratio biomass stability was attained. Settling property of sludge was good. Urea removal was more or less complete at 2 days detention period at the above C:N:P ratio.

The whole of Phase 2 consisted of 3 parts. In Part 1, biomass concentration was in the range of 4800-5400 mg/l in all reactors. No pH control was done. In Part 2, the biomass concentration was maintained as in Part 1. However, in Reactors 2 and 3 the biomass concentration was maintained in the range of 1500-1600 mg/l. This change was done due to the low efficiency in Reactors 2 and 3 in Part 1. In Part 2 also no pH control was done. In Part 3, which was exactly

like the Part 2, pH was maintained at 8.0-8.3 in addition. The effluent of Reactor 1 was used as influent of Reactor 2. To this effluent additional quantity of organic carbon was supplemented so as to maintain the C:N:P ratio at 10:10:1. To the effluent of Reactor 2, similar organic carbon addition was done. Each part of experiments is discussed in detail in the following sections.

6.6.1. Phase 2 - Part 1: Ammonia and Urea Removal Study with Batch Reactors in Series; Biomass Concentration 5000 mg/l and No pH Adjustment:

The biomass concentrations in all the reactors were maintained at 4[illegible]-5400 mg/l. pH was not adjusted. To the settled effluent of Reactor 1, organic carbon was added to make up the C:N:P ratio 10:10:1. This was possible from the knowledge of the effluent total N concentration and COD concentration. After steady state condition was achieved, readings were taken. This is explained in Section 5.4.1. Complete details of experimental results are presented in Appendix E.1. A typical set of relevant readings are presented in Table 32.

It can be seen from the table that 33 percent of total N was removed in Reactor 1. The effluent of Reactor 1 containing 645 mg/l total N was the influent in Reactor 2. In Reactor 2, total N removal efficiency was 29 percent. The resulting effluent contained 460 mg/l of total N. The effluent from Reactor 3 contained total N of 380 mg/l. The reason for the lower efficiencies in Reactors 2 and 3 could be due to excessive

Table 32 - Biomass Concentration, Total N and COD Removal Efficiencies in Phase 2 - Part 1 Study, Temperature 28-37°C

Reactor 1			Reactor 2			Reactor 3		
Biomass concentration mg/l	Total N removal efficiency %	COD removal efficiency %	Biomass concentration mg/l	Total N removal efficiency %	COD removal efficiency %	Biomass concentration mg/l	Total N removal efficiency %	COD removal efficiency %
5200	33	95	5100	29	87	4900	17	97
Influent total N = 970 mg/l			Influent total N = 645 mg/l			Influent total N = 460 mg/l		
COD = 2650 mg/l			COD = 2000 mg/l			COD = 1470 mg/l		
Initial pH = 8.1			Initial pH = 6.7			Initial pH = 6.2		
Final pH = 6.7			Final pH = 6.2			Final pH = 5.9		

biomass at lower pH. As organic carbon was added only in relation to the total N in the effluent, this organic carbon might have been insufficient to support such a large biomass. This experiment revealed an important fact that high biomass concentrations in Reactors 2 and 3 were of no use in enhancing the total N removal efficiency. Hence a biomass concentrations of 1500-1600 mg/l were used in Reactors 2 and 3 in the subsequent experiments.

6.6.2. Phase 2 - Part 2: Ammonia and Urea Removal Study with Batch Reactors in Series; Reduced Biomass Concentrations in Reactors 2 and 3 and No pH Adjustment:

The initial biomass concentrations in Reactors 2 and 3 were of the order of 1500-1600 mg/l. However, the biomass concentration in Reactor 1 was maintained at 4800-5400 mg/l. Table 33 shows a typical set of readings.

Table 33 - Biomass Concentration, Total N and COD Removal Efficiencies in Phase 2 - Part 2 Study, Temperature 29-38°C

Reactor 1			Reactor 2			Reactor 3		
Biomass concentration mg/l	Total N removal efficiency %	COD removal efficiency %	Biomass concentration mg/l	Total N removal efficiency %	COD removal efficiency %	Biomass concentration mg/l	Total N removal efficiency %	COD removal efficiency %
5200	43	89	1600	30	98	1400	30	97
Influent total N = 985 mg/l			Influent total N = 565 mg/l			Influent total N = 405 mg/l		
COD = 2650 mg/l			COD = 1920 mg/l			COD = 1470 mg/l		
Initial pH = 7.9			Initial pH = 6.3			Initial pH = 5.7		
Final pH = 6.2			Final pH = 5.9			Final pH = 5.5		

It can be observed that there was considerable increase in total N removal efficiency in Reactor 3. However, the efficiency in Reactor 2 did not improve much. Biomass concentration was

of the order of 1400-1600 mg/l each in Reactors 2 and 3. Overall total N removal efficiency (72 percent) in this case was better than that obtained in the earlier experiment. pH was not controlled in this part also. Hence in Phase 2 - Part 3, the effect of pH on the total N removal efficiency was studied. The enhancement of total N removal efficiency at pH 8.0-8.3 was earlier studied in a single batch reactor in Phase 1 - Part 6.

6.6.3. Phase 2 - Part 3: Ammonia and Urea Removal Study with Batch Reactors in Series; Reduced Biomass Concentrations in Reactors 2 and 3 and with pH at 8.0-8.3:

The procedure adopted in this experiment is described in Section 5.4.3. The only difference between this experiment and that done in Phase 2 - Part 2 was that in this case pH was maintained in the range of 8.0-8.3 by adding Na_2CO_3. The results obtained are presented in Appendix E.3. One typical set of readings is presented in Table 34.

It can be clearly seen that pH drop was observed. However, it was maintained at 8.0-8.3 as explained earlier. The total N removal efficiency was very much higher, when compared to that in Phase 2 - Part 2 study. The overall total N removal efficiency (92 percent) was considerably higher. This was due to higher pH maintained in the range of 8.0-8.3. At higher pH, ammonia might have passively entered into the cells as explained in earlier sections.

Table 34 - Biomass Concentration, Total N and COD Removal Efficiencies in Phase 2 - Part 3 Study, Temperature 29-40°C

Reactor 1			Reactor 2			Reactor 3		
Biomass concentration mg/l	Total N removal efficiency %	COD removal efficiency %	Biomass concentration mg/l	Total N removal efficiency %	COD removal efficiency %	Biomass concentration mg/l	Total N removal efficiency %	COD removal efficiency %
5200	52	91	1600	55	100	1500	62	96
Influent total N = 970 mg/l			Influent total N = 470 mg/l			Influent total N = 210 mg/l		
COD = 2650 mg/l			COD = 1840 mg/l			COD = 870 mg/l		
Initial pH = 8.2			Initial pH = 8.3			Initial pH = 8.1		
Final pH = 7.1			Final pH = 6.3			Final pH = 6.0		

When all the organic carbon fed to the 3 reactors were added up, then C:N:P ratio came up to 20:10:1. Considering the total N removal efficiency of a single reactor as in Phase 1 - Part 4, the total N removal efficiency at a C:N:P ratio of 20:10:1 was 62 percent, whereas in this case of 3 batch reactors it was 72 percent. Temperature range for the Phase 1 - Part 4 study was more favourable (24-31°C) than that prevailing during Phase 2 - Part 2 study (29-38°C). At higher night temperatures, efficiency of nutrient assimilation and growths goes down (Davis et.al., 1953). The higher day time temperature of

Phase 2 - Part 2 study might also have affected the efficiency considerably. So it can be concluded that 3 batch reactors in series prove to be much superior to a single batch reactor under identical conditions.

6.6.4. Evaluation of k:

The experiment was conducted for the evaluation of k, the total N removal velocity constant. This is described in detail in Section 5.4.4. k is the first order reaction constant in equations 4.18 and 4.19. Tables 35 and 36 show the experimental results obtained for various detention times.

The value of $k(\overline{C})$ was obtained from the equation 4.18. Then the value of $k(\overline{C})$ was plotted on Y axis against the corresponding values of $\overline{C}$ along X axis. Figures 46 and 47 give the values of k for reactors with pH control and without pH control respectively.

The value of k for reactor with no pH control was found to be 0.40, whereas it was 0.67 for that with pH maintained in the range of 8.0-8.3. It can be seen from this study that the value of reaction constant for reactor with pH control (8.0-8.3) was much higher than that for reactor with no pH control. These values would be helpful in calculating the effluent concentration within the range of detention times studied.

Table 35 - Evaluation of Reaction Constant, k - with No pH Control, Temperature 29-38°C

Tank volume litres V	Detention time days T	Flow rate l/day Q	Influent total N concentration mg/l C_o	Steady state effluent total N concentration mg/l $\overline{C}$	Calculated value of rate of reaction mg/l/day $k(\overline{C})$
2.5	2	1.25	975	565	205
2.5	3	0.83	970	525	149
2.5	4	0.63	985	475	128
2.5	6	0.41	975	430	91

Table 36 - Evaluation of Reaction Constant, k - with pH at 8.0-8.3, Temperature 29-38°C

Tank volume litres V	Detention time days T	Flow rate l/day Q	Influent total N concentration mg/l C_o	Steady state effluent total N concentration mg/l $\overline{C}$	Calculated value of rate of reaction mg/l/day $k(\overline{C})$
2.5	2	1.25	970	470	250
2.5	3	0.83	985	425	187
2.5	4	0.63	970	325	162
2.5	6	0.41	975	300	112

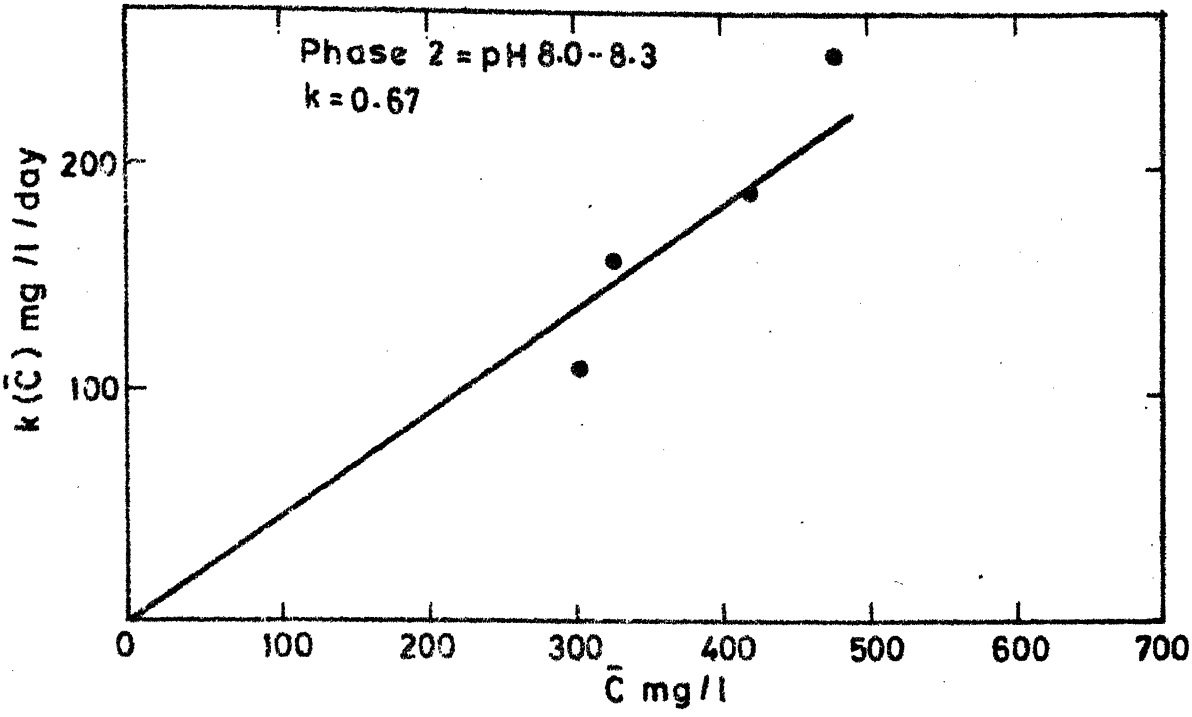


FIG. 46 EVALUATION OF REACTION CONSTANT

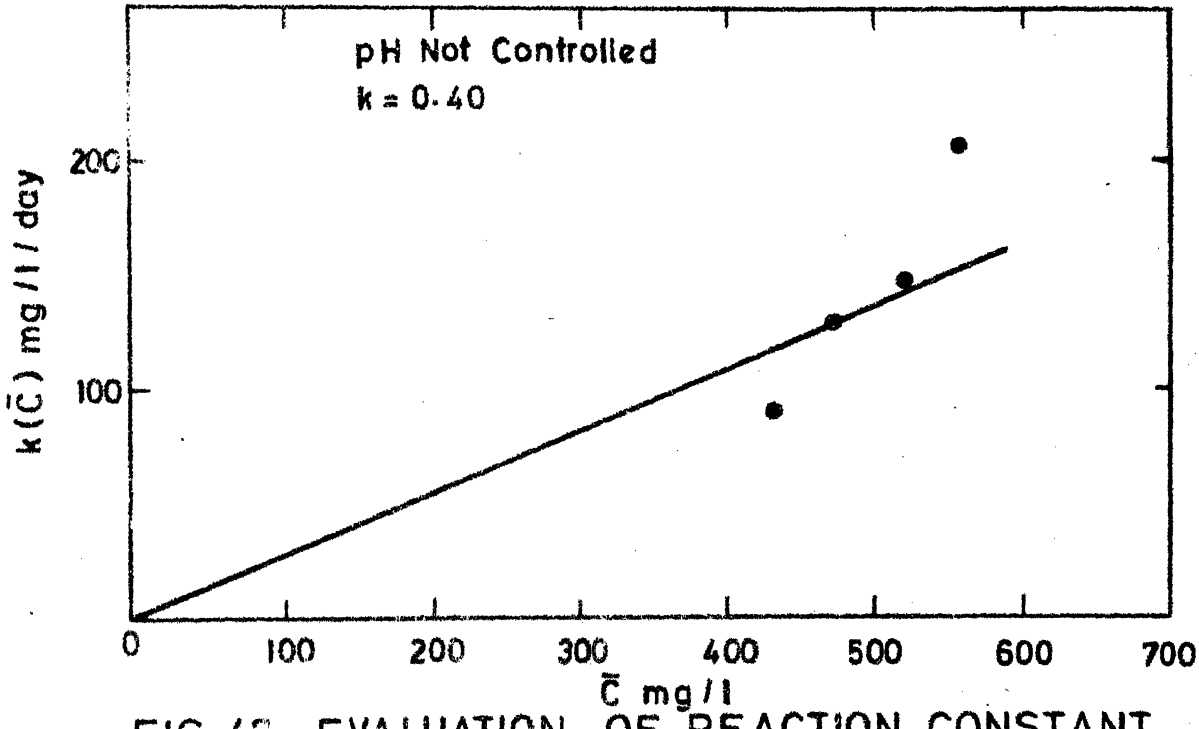


FIG. 47 EVALUATION OF REACTION CONSTANT

6.7. Phase 3: Continuous Flow Single Reactor Study:

The equations for the determination of biokinetic constants are already derived in Section 4.5.1. The equations are:

$$X_1 = \frac{Y(S_o - S_1)}{(1 + kd\theta)} \qquad 6.1$$

and

$$S_1 = \frac{K_s(1 + kd\theta)}{\theta\bar{\mu} - (1 + kd\theta)} \qquad 6.2$$

Rearranging and inverting equations 6.1 and 6.2, we get

$$\frac{S_o - S_1}{X_1} = \frac{kd\theta}{Y} + \frac{1}{Y} \qquad 6.3$$

and

$$\frac{\theta}{1 + kd\theta} = \frac{K_s}{\bar{\mu}S} + \frac{1}{\bar{\mu}} \qquad 6.4$$

where S_o = Total influent N concentration (mg/l)

S_1 = Total effluent N concentration (mg/l)

X_1 = Biomass concentration at steady state condition (mg/l)

kd = Microorganism decay rate (day^{-1})

Y = Yield coefficient (mg of biomass/mg total N removed)

$\bar{\mu}$ = Maximum specific growth rate (day^{-1})

S = Total N concentration surrounding the biomass (mg/l)

K_s = Saturation constant defined as substrate concentration at which = $\frac{\bar{\mu}}{2}$ (mg/l)

The procedure adopted is described in detail in Section 5.5. The steady state values obtained at various SRTs are shown in Table 37. pH in this experiment was maintained at 8.0-8.3 throughout. The liquid detention time was 1 day throughout the experiment. SRTs were varied.

Table 37 - Biomass Concentration, Total N Concentration in Influent and Effluent, Settled Sludge Value and Biomass Concentration at Steady State Conditions, Temperature 15-28°C

SRTs days θ	Average biomass concentration mg/l X_1	Influent total N concentration mg/l S_o	Effluent total N concentration mg/l S_1	$\frac{S_o - S_1}{X_1}$	Settled sludge volume ml	Effluent biomass concentration mg/l
10	4500	985	300	0.1522	100	
8	4000	975	375	0.1500	80	
6	4000	970	400	0.1425	60	60
4	3600	975	420	0.1541	60	100

From the above data in the table values of $\frac{S_o - S_1}{X_1}$ for various SRTs were calculated. These values were plotted along the Y axis with corresponding values of θ along X axis. A straight line was drawn through the points to meet the Y axis. This is shown in Figure 48. The intercept on the Y axis is the value of $\frac{1}{Y}$. From this value, the value of Y has been

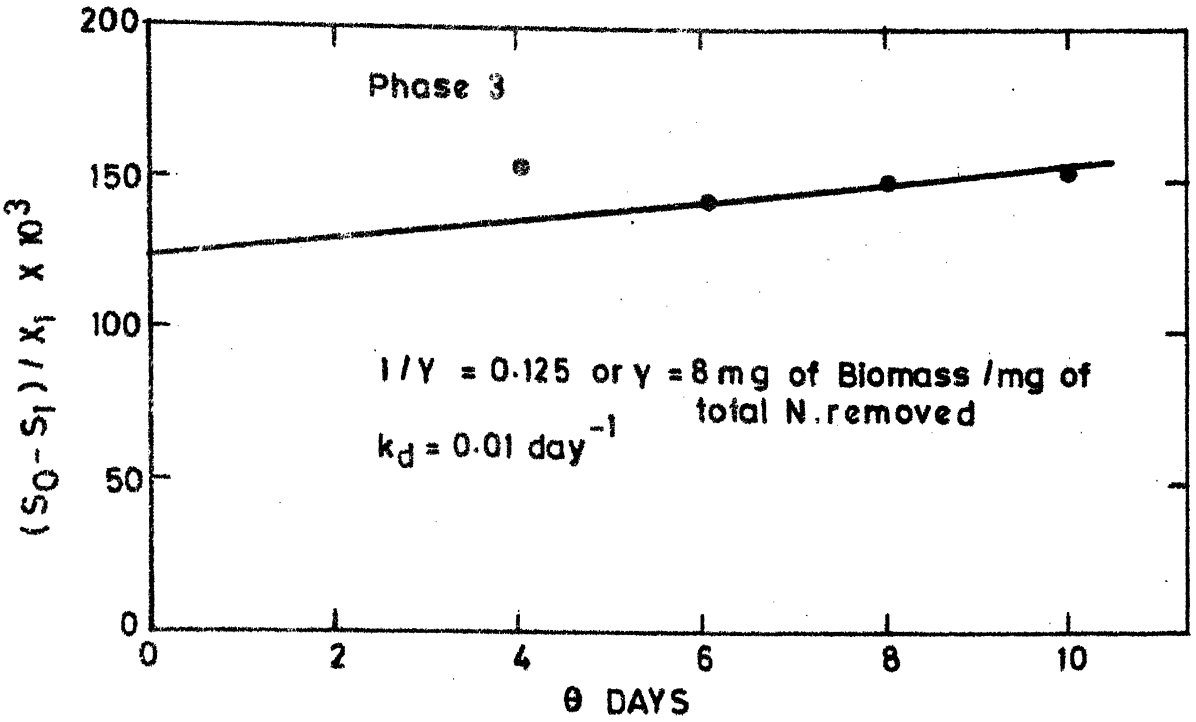


FIG. 48 EVALUATION OF Y AND k_d

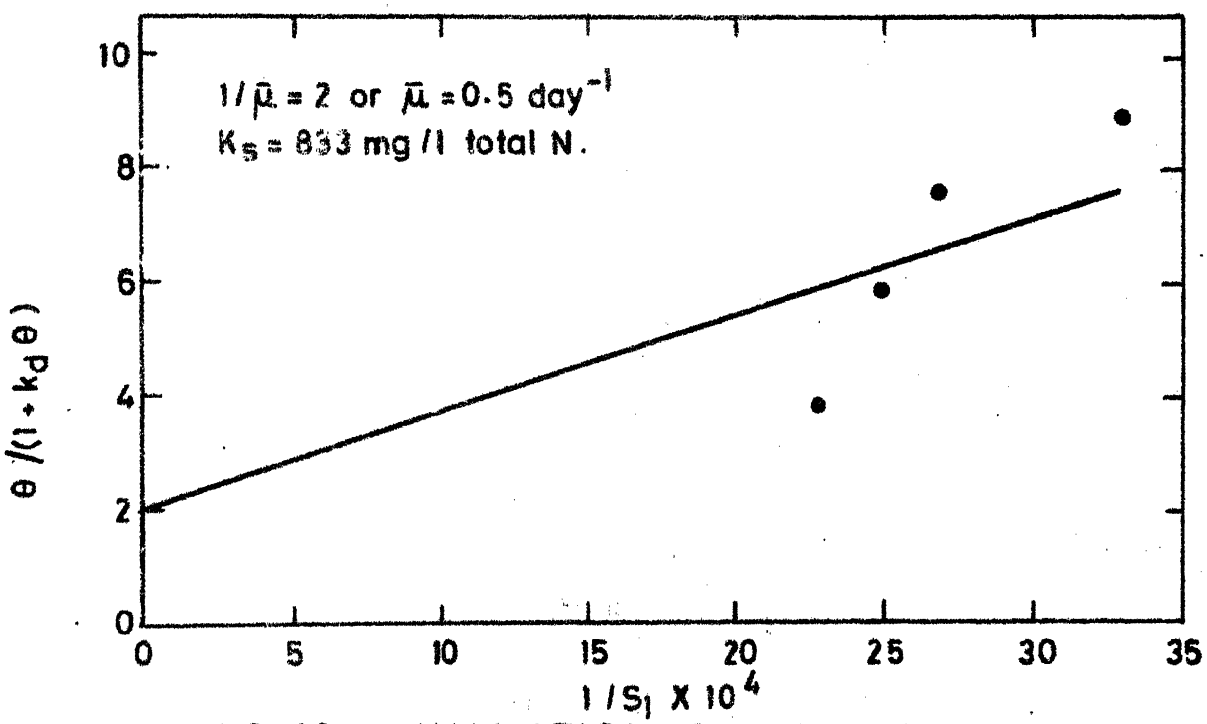


FIG. 49 EVALUATION OF $\bar{\mu}$ AND K_s

found to be 8.0 mg biomass/mg of total N removed. In other words 12.5 percent of total N has been removed and incorporated in the biomass.

The slope of the line was found to be 0.0025. The slope is also equal to $\frac{kd}{Y}$. From the known value of Y and the value of slope, kd was determined. The value of kd so obtained was 0.01 day^{-1}.

Then the reciprocal values of S_1, the total N concentration surrounding the biomass and the values of $\frac{\theta}{(1 + kd\theta)}$ are calculated for various SRTs. These values are shown in Table 38. Under steady state conditions, S_1 is nothing but the effluent total N concentration.

Table 38 - Values of $\frac{1}{S_1}$ and $\frac{\theta}{(1 + kd\theta)}$ for the Determination of $\bar{\mu}$ and K_s.

S_1	$\frac{1}{S_1}$	θ	$1 + kd\theta$	$\frac{\theta}{(1 + kd\theta)}$
300	0.0033	10	1.10	9.09
375	0.0027	8	1.08	7.41
400	0.0025	6	1.06	5.66
420	0.0023	4	1.04	3.85

Then the values of $\frac{1}{S_1}$ along X axis and corresponding values of $\frac{\theta}{(1 + kd\theta)}$ along Y axis are plotted and a straight line obtained with an intercept on the Y axis. This is shown in Figure 49. The value of the intercept on the Y axis is $\frac{1}{\bar{\mu}}$. It was found to be 2.0. In other words $\bar{\mu}$ was found to be 0.5 day^{-1}. K_s was determined from the slope of the line. The value so obtained was 833 mg/l.

It has been reported by Pearson (1968) and Metcalf and Eddy (1974), that $\bar{\mu}$ for mixed culture of bacterial aerobic system is 3.7 day^{-1} for domestic waste and 2.4 day^{-1} for skim milk. Golueke et.al. (1959) reported that the value for algae is 0.24 day^{-1} at an algal concentration of 4000-4500 mg/l. However, when the algal concentration was 316 mg/l, $\bar{\mu}$ was as high as 1.82 day^{-1}. Environmental conditions were different from those existing in the present study. The value of $\bar{\mu}$ obtained for algal-bacterial process in the present study is 0.5 day^{-1}. This being a mixture of algae and bacteria and the treating high nitrogenous wastes under carbon stressed conditions, this rate seems to be reasonable. Moreover, nitrifiers, though to a small extent, constitute the microflora. Their growth rate is reported to be too small. Since the present system is a combination of algae and bacteria, the maximum specific growth rate of 0.5 day^{-1} seems to be logical as it is in-between that of algae and bacteria. No comparison can be made from the literature as this a unique system which is being followed for treating high nitrogenous wastes.

6.8. Modified Stoichiometric Equations and Luxury Uptake of Nitrogen for Continuous Flow Single Reactor System:

The values obtained from the continuous flow single reactor study is shown in Table 39.

Table 39 - Biomass Concentration, Influent Total N and Effluent Total N Concentrations in Continuous Flow Single Reactor, Temperature 15-28°C

SRTs days θ	Average biomass concentration mg/l X_1	Influent total N concentration mg/l S_o	Effluent total N concentration mg/l S_1	Total N removed by air-stripping mg/l S_x	Total N utilised by biomass mg/l
10	4500	985	300	20	665
8	4000	975	375	20	580
6	4000	975	400	20	550
4	3600	975	420	20	535

The general method followed in the development of stoichiometric equation is on the same lines as followed by Sherrod et.al. (1975) for the treatment of organic matter by bacterial flora. It has been observed that the algae/bacteria ratio by weight was in the range of 1.3-2.0 in many of the batch reactors. In the continuous flow single reactor it was found to be in the range of 1.5-2.0. Hence an average ratio of 1.7 is quite reasonable in calculating the molecular weight ratio.

If x is the molecular proportion of algae in the biomass, then

$$\text{Bacterial proportion} = (1 - x)$$

Then $\frac{\text{Algae}}{\text{Bacteria}}$ ratio $= \frac{x}{(1 - x)}$ by molecular weight

The $\frac{\text{Algae}}{\text{Bacteria}}$ weight ratio can be obtained by putting the formulae for algae and bacteria as follows:

$$\frac{x(C_{5.7}H_{9.8}O_{2.3}N)}{(1 - x)C_5H_7O_2N} = 1.7 \qquad 6.5$$

Therefore $\frac{x \times 129}{(1 - x)\ 113} = 1.7$. Solving this equation, we get

$$x = 0.598 \quad \text{or} \quad x = 0.6$$

and $$(1 - x) = 0.4$$

We have

$$Y = \frac{y\ (\text{Molecular weight of biomass})}{\text{Total N utilised by the biomass}} \qquad 6.6$$

where Y = Yield coefficient

y = Number which, when multiplied by the composite biomass would yield the stoichiometric equation.

The denominator in the above equation depends on the SRTs.

The general form of equation, when glucose, ammonia and carbon dioxide are substrates, is:

$$x.C_6H_{12}O_6 + y.NH_3 + z.CO_2 + a.H_2O \longrightarrow$$

$$y(0.6\ C_{5.7}H_{9.8}O_{2.3}N + 0.4\ C_5H_7O_2N) + b.O_2 \qquad 6.7$$

y can be obtained from the equation 6.6. From Phase 3 study, the value of Y was obtained as 8.0 mg of biomass/mg of total N removed. Hence y is calculated for all the 4 SRTs and discussed. This would give the biomass concentration, total N removal efficiency and the contribution of N luxury uptake towards this. This would help to select the optimum SRT for the minimum biomass and maximum N removal efficiency.

6.8.1. SRT of 10 Days:

$$Y = \frac{y(0.6\ C_{5.7}H_{9.8}O_{2.3}N + 0.4\ C_5H_7O_2N)}{665}$$

$$= \frac{y(0.6 \times 129 + 0.4 \times 113)}{665} = \frac{122.6\ y}{665}$$

$$\text{or} \quad y = \frac{665 \times 8}{122.6} = 43.39.$$

Hence the stoichiometric equation can be written as:

$$14.47\ C_6H_{12}O_6 + 43.39\ NH_3 + 148.37\ CO_2 + 36.4\ H_2O \longrightarrow$$

$$26.03\ C_{5.7}H_{9.8}O_{2.3}N + 17.36\ C_5H_7O_2N + 162.69\ O_2 \qquad 6.8$$

Assuming that all glucose was utilised by bacteria and only CO_2 was utilised by algae, the stoichiometric can be partly completed by balancing carbon. Then hydrogen is balanced

and finally oxygen. From the above equation the biomass that could be produced is calculated as:

$$\text{Biomass} = 26.03\ C_{5.7}H_{9.8}O_{2.3}N + 17.36\ C_5H_7O_2N$$

$$= 26.03 \times 129 + 17.36 \times 113 = 5320 \text{ mg}$$

However, in the reactor the biomass concentration at an SRT of 10 days was 4500 mg/l. The sludge wasted per day was 450 mg/l. Hence total biomass in the reactor works out to be 4950 mg/l.

This indicates that a smaller quantity of biomass, than the stoichiometric quantity could remove the total N as given above. From Phase 1 - Part 7 study it was clearly shown that there exists a luxury uptake of nitrogen in the biomass. So if a smaller quantity of biomass than the stoichiometric quantity is capable of removing certain quantity of total N, it could do so only by luxury uptake. Hence this calculation proves indirectly the existence of such a phenomenon in the algal-bacterial system.

6.8.2. SRT of 8 Days:

$$Y = \frac{y(0.6\ C_{5.7}H_{9.8}O_{2.3}N + 0.4\ C_5H_7O_2N)}{580}$$

or $$y = \frac{580 \times 8}{122.6} = 37.85 \text{ (As done earlier).}$$

The stoichiometric equation can be written as:

$$12.62\ C_6H_{12}O_6 + 37.85\ NH_3 + 129.45\ CO_2 + 31.78\ H_2 \longrightarrow$$
$$22.71\ C_{5.7}H_{9.8}O_{2.3}N + 15.14\ C_5H_7O_2N + 141.95\ O_2 \qquad 6.8$$

Total biomass produced according to this equation is:

$$= 22.71 \times 129 + 15.14 \times 113 = 4640 \text{ mg}$$

However, at an SRT of 8 days, the average biomass concentration in the reactor was 4000 mg/l and biomass wasted was 500 mg/l. Hence the total biomass was 4500 mg/l. Here also the stoichiometric amount of biomass is more than actual biomass. Here too the N removal might have been by luxury uptake mechanism to a certain extent.

6.8.3. SRT of 6 Days:

$$Y = \frac{y(0.6\ C_{5.7}H_{9.8}O_{2.3}N + 0.4\ C_5H_7O_2N)}{550}$$

$$\text{or} \quad y = \frac{550 \times 8}{122.6} = 35.89$$

The stoichiometric equation in this case is:

$$11.97\ C_6H_{12}O_6 + 35.89\ NH_3 + 122.72\ CO_2 + 30.1\ H_2O \longrightarrow$$
$$21.53\ C_{5.7}H_{9.8}O_{2.3}N + 14.36\ C_5H_7O_2N + 134.56\ O_2 \qquad 6.9$$

Total biomass produced as per the above equation is:

$$= 21.53 \times 129 + 14.36 \times 113 = 4400 \text{ mg}$$

The biomass in the reactor at SRT of 6 days was 4000 mg/l. The sludge wasted was 650 mg/l. Hence the total biomass works out to be 4650 mg/l. This is more than the stoichiometric amount of biomass.

6.8.4. SRT of 4 Days:

$$Y = \frac{y(0.6\ C_{5.7}H_{9.8}O_{2.3}N + 0.4\ C_5H_7O_2N)}{535}$$

or $$y = \frac{535 \times 8}{122.6} = 34.91$$

And the stoichiometric equation can be written as:

$$11.63\ C_6H_{12}O_6 + 34.91\ NH_3 + 119.42\ CO_2 + 29.37\ H_2O \longrightarrow$$
$$20.95\ C_{5.7}H_{9.8}O_{2.3}N + 13.96\ C_5H_7O_2N + 130.94\ O_2 \qquad 6.10$$

Total biomass produced as per this equation is

$$= 20.95 \times 129 + 13.96 \times 113 = 4280 \text{ mg}$$

However, the biomass in the reactor was 3600 mg/l. The sludge wasted was 900 mg/l. Hence the total biomass in the reactor was 4500 mg/l. Here too the actual biomass in the reactor was more than the calculated amount for the removal of 535 mg/l of total N.

From the above calculations and the actual performance of the reactor, it can be seen that, when SRTs were 8-10 days, more of total N was removed, with comparatively less biomass concentrations. However,at shorter SRTs, the total N removal

efficiencies were less in terms of unit weight of biomass. Hence SRTs of 8-10 days are preferable for the efficient working of a continuous flow single reactor. In the batch reactor studies, reported in Section 6.2.7, the same trend was observed. The quantity of total N incorporated, and therefore removed, was more at longer detention times. However, from the point of view of COD and effluent biomass concentrations, shorter detention times were found suitable in batch reactors. The effluent biomass and COD concentrations were least at SRTs of 8-10 days in the continuous flow single reactor system. Hence longer SRTs are preferred in such cases.

6.9. Summary of the Optimum Operating Conditions for the Continuous Flow Single Reactor:

The optimum conditions for efficient total N removal in a continuous flow single reactor are given in Table 40.

Table 40 - Optimum Conditions for the Operation of Continuous Flow Single Reactor

No.	Parameters	Quantities
1	Liquid detention time, T	1 day
2	Solids retention time, θ	8-10 days
3	Maximum efficiency of total N removal	70 percent
4	Maximum specific growth rate, $\bar{\mu}$	0.5 day^{-1}
5	Microorganism decay rate, kd	0.01 day^{-1}
6	Yield coefficient, Y	8.0 mg biomass/ mg total N removed

7. STUDY OF FLOCCULATING ALGAL-BACTERIAL SYSTEM USING MILK WASTE (WHEY) AS ORGANIC CARBON SOURCE

7.1. Milk Waste, Good Source of Organic Carbon:

Whey from cheese manufacturing units contains BOD to an extent of 32000 mg/l, according to Nemerow (1971). However, this BOD depends on the dilution and the efficiency of cheese making. In this study, undiluted whey was obtained from a private cheese manufacturing company at Kanpur. The COD was found to be 64000 mg/l. This was tried in the laboratory as a substitute for glucose.

7.2. Experimental Study:

A set of batch study was conducted to explore the suitability of whey as a source of organic carbon in the flocculating algal-bacterial system for treating high nitrogenous wastes. Due to lack of time and laboratory limitations, a batch study alone was attempted. Two reactors, identical with the previous ones, were set up for the experimental study. Biomass obtained from the previous experiment was washed well before using the same during the experiment. Initial biomass concentrations were approximately 4000 mg/l.

Glucose was used as a source of organic carbon in Reactor 1, while whey was used as organic carbon source in Reactor 2. The feed to both the reactors contained 700 mg/l ammonia N and

300 mg/l urea N. The C:N:P ratio adopted here was 10:10:1. The procedure adopted was exactly the same as described in Phase 1 - Part 7 study. Acclimatisation was done as in earlier batch reactor studies. It was found that meaningful informations were obtained up to a detention time of 4 days from batch reactor studies. So this experiment was carried out for 4 days after acclimatisation.

The experiment was started, after steady state condition, as revealed by biomass and total N removal, was achieved. Then fresh feeds were introduced once only. Glucose was the organic carbon source in Reactor 1, whereas it was whey in Reactor 2. In either cases, all other ingredients added, such as ammonia, urea, phosphate and sodium carbonate were the same. pH was maintained in the range of 8.0-8.3 everyday. Readings were taken soon after feeding and at the end of 1, 2 and 4 days.

7.3. Results and Discussion:

The data after feed are presented in Table 41. It can be observed from the table that the biomass increase was slightly higher in Reactor 2 than in Reactor 1. The maximum biomass concentrations in Reactor 1 was 4500 mg/l on the 2nd day. The corresponding figure in Reactor 2 was 5100 mg/l. So, from the point of view of biomass increase rate, whey waste seems to be better than glucose. Another point to be noted is that the algae/bacteria ratio in Reactor 1 was ranging between 1.3-1.8.

Table 41 - Results of Experiment with Glucose and Whey as Organic Carbon Source and C:N:P Ratio of 10:10:1, pH at 8.0-8.3, Temperature 12-25°C

Reactors	Biomass concentration mg/l		Algae concentration mg/l		Algae/bacteria ratio		Influent characteristics									
							NH_3-N mg/l		Urea N mg/l		COD mg/l		pH		Alkalinity mg/l	
Days	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
0	4200	4300	2600	2800	1.67	1.86	650	650	320	320	2750	2900	8.1	8.2	480	480
1	4300	4600	2600	3000	1.53	1.87										
2	4500	5100	2600	3600	1.30	2.40										
4	4200	5000	2700	3600	1.80	2.57										

Reactors	Effluent characteristics																	
	NH_3-N mg/l		Urea N mg/l		NO_2-N mg/l		NO_3-N mg/l		COD mg/l		pH		Alkalinity mg/l		Total N removal percent		COD removal percent	
Days	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
0																		
1	425	350	100	50	10				400	480	6.8	6.8	240	180	45	59	85.5	88.5
2	300	275			20	10	5	5	400	420	6.6	6.6	180	140	65	70	85.5	85.5
4	275	250			20	20	10	15	220	260	7.0	7.1	120	110	68	70	92.0	91.0

However, the ratio was 1.86-2.57 in Reactor 2. The algal biomass concentration was much higher in Reactor 2. Settling values and effluent biomass concentrations were more or less comparable in both the reactors. The settling values were 60 ml and 150 ml in Reactors 1 and 2 respectively. The biomass concentration in the effluents were 70 mg/l and 160 mg/l in Reactors 1 and 2 respectively.

The total N removal efficiency was 45 percent on the 1st day and went up to 68 percent on the 4th day in Reactor 1. The above values were 59 percent on the 1st day and 70 percent on the 4th day in Reactor 2. The total N removal efficiency was more in Reactor 2 compared to that in Reactor 1. This might be possibly due to the presence of larger quantity of algae in Reactor 2. COD removal efficiency in Reactor 1 was slightly better than that in Reactor 2. The efficiencies ranged from 85.5 percent on the 1st day to 92 percent on the 4th day. The corresponding values were 83.5 percent and 91 percent respectively in Reactor 2.

7.4. Conclusions:

So it can be concluded that milk waste (whey) can be successfully used as a source of organic carbon in flocculating algal-bacterial system treating high nitrogenous wastes. From the above study, it can be observed that whey wastes are equally good in the total N removal efficiency as a source of

organic carbon as is glucose. This is of economic and practical importance in the treatment of high nitrogenous wastes as well as milk wastes. Further studies on continuous flow reactors, using whey as an organic carbon source for the treatment of high nitrogenous wastes, would reveal more useful informations.

8. CONCLUSIONS

All conclusions arrived at, in this thesis, are based upon the data collected during the 3 phases of experimental studies and also upon the discussion made in Chapters 6 and 7.

1. Flocculating algal-bacterial system can be successfully adopted for the treatment of ammonia N concentrations up to 1000 mg/l with organic carbon supplement. The efficiency obtained is of the same order when ammonia N (700 mg/l) and urea N (300 mg/l) are present instead of ammonia N (1000 mg/l) alone.
2. Continuous maintenance of pH in the range of 8.0-8.3 is effective in enhancing the total N removal efficiency.
3. Carbon dioxide does not significantly improve the total N removal efficiency.
4. Since the requirement of organic carbon is large, sewage is not suitable as a substitute of organic carbon for treating undiluted high nitrogenous wastes. The experiment with milk wastes (whey) shows that this industrial waste would be a very good substitute for glucose as a source of organic carbon for treating high nitrogenous wastes. Incidentally, milk waste also gets treated in the process.
5. Flocculating algal-bacterial system appears to incorporate the advantages of both activated sludge

system and algal system, with enhanced total N removal efficiency and good biomass settling characteristics.

6. Continuous flow single reactor experiment proves that flocculating algal-bacterial system works quite efficiently for a total N concentration of 1000 mg/l at a C:N:P ratio of 10:10:1 and a pH range of 8.0-8.3.

7. Comparatively low concentrations of nitrite N and nitrate N are observed in the system. It was of the order of 0-70 mg/l in many of the experiments.

8. Total N removal efficiency ranges from 31-82 percent depending upon the C:N:P ratio, temperature and pH. The higher the C:N:P ratio, the higher is the total N removal efficiency.

   If a C:N:P ratio, less than the stoichiometric ratio, is to be used to reduce the organic carbon supplement, then the present study shows that a C:N:P ratio of 10:10:1 is the optimum, based on the total N and COD removal efficiencies and biomass flocculation and settleability.

9. The optimum algae/bacteria ratio seems to be 1.3-2.0 at a total N feed concentration of 1000 mg/l and a C:N:P ratio of 10:10:1.

10. A detention time of 2 days seems to be suitable in batch reactors, from the point of view of total N and COD removal efficiencies and biomass flocculating and

settling characteristics. However, in continuous flow single reactor, SRT seems to be 8-10 days for the maximum total N removal efficiency.

11. Luxury uptake of nitrogen takes place in a flocculating algal-bacterial system treating high nitrogenous wastes. This happens under carbon stressed conditions and at detention times of 4-6 days in batch reactors. A maximum uptake of nitrogen to the extent of 16 percent of the biomass weight seems to occur in the above cases. However, in the continuous flow reactors, luxury uptake seems to be not of a high order. Still, luxury uptake of nitrogen takes place in continuous flow reactors at SRTs of 8-10 days.

12. For additional removal of nitrogen from high nitrogenous wastes, a set of about 3 reactors working in series is recommended. If, in this system, pH is maintained in the range of 8.0-8.3, the total N removal efficiency can easily reach up to 92 percent. If pH is not controlled, the study indicates, that the total N removal efficiency would be of the order of 72 percent.

13. In a continuous flow single reactor, a liquid detention time of 1 day and SRT of 8-10 days seems to be the optimum from the points of view of total N removal efficiency (62-70 percent), biomass flocculating and settling characteristics and effluent biomass concentrations.

14. From the continuous flow single reactor studies, treating high nitrogenous wastes by flocculating algal-bacterial system, the process biokinetic constants and other factors are given below:

Liquid detention time, T	1 day
Solids retention time, θ	8-10 days
Maximum efficiency of total N removal	70 percent
Maximum specific growth rate, $\bar{\mu}$	0.5 day^{-1}
Microorganism decay rate, kd	0.01 day^{-1}
Yield coefficient, Y	8.0 mg biomass/ mg total N removed

15. Stoichiometric equations have been derived from the values of Y and total N incorporated in the cells at various SRTs.

9. ENGINEERING SIGNIFICANCE AND SUGGESTION FOR FUTURE WORK

9.1. Engineering Significance:

Treatment of high nitrogenous wastes by flocculating algal-bacterial system offers an elegant method, in which urea and ammonia together can be treated very efficiently. The removed nitrogen is incorporated in the biomass, which can be used as a good manure after digestion or composting. Compared to all other methods of nitrogen removal from high nitrogenous wastes, flocculating algal-bacterial system alone utilises and conserves the nitrogen usefully. So from the point of view of conservation of water and nitrogen, this method offers a promising technique in the treatment of high nitrogenous wastes.

9.2. Suggestion for Future Work:

It may be noted that whey was responding favourably as a source of organic carbon in the flocculating algal-bacterial system. The effect of higher C:N:P ratio on the nitrogen removal efficiency is worth studying, with milk wastes and similar other wastes. Some of the industrial wastes, that can be tried as a source of organic carbon or starch wastes, cannery wastes etc. Pilot plant study must be conducted, before translating the results obtained from the present results, in actual operation. However, as the laboratory studies have revealed various important parameters and biokinetic constants, this would serve as a guide in the study of pilot plant studies.

REFERENCES

Adams, C.E., Jr., and Eckenfelder, W.W., Jr., (1977). "Nitrification Design Approach for High Strength Ammonia Wastewater", J. Water Poll. Cont. Fed., 49, 3, 413.

Alagarswamy, S.R., (1967). "Effect of Influent pH Value on Performance of Oxidation Pond", J. Env. Health, 9, 57.

Anthonisen, A.C., Loehr, R.C., Prakasam, T.B.S., and Srinath, E.G., (1976). "Inhibition of Nitrification by Ammonia and Nitrous Acid", J. Water Poll. Cont. Fed., 44, 6, 1135.

Arnon, D.I., (1960). "The Role of Light in Photosynthesis", Scientific American, 203, 104.

Bartsch, A.F., (1961). "Algae as a Source of Oxygen in Waste Treatment", J. Water Poll. Cont. Fed., 33, 3, 239.

Bhalerao, S.B., and Alagarswamy, S.R., (1973). "Treatment Alternatives and Cost Studies for Liquid Effluents in Fertiliser Industry", Indian J. Env. Health, 15, 4, 329.

Bhattacharrya, G.C., Roy, G.S., and Dutta, B.K., (1966). "Investigation in the Use of Algae for Removing Nitrogen from Nitrogenous Industrial Wastes", Technology, 3, 3, 135.

Bhattacharrya, G.S., Roy, G.S., and Dutta, B.K., (1968). "Investigation into the Use of Algae for Removing Ammonia Nitrogen from Nitrogenous Industrial Wastes", Technology, 5, 1, 31.

Bhattacharrya, G.S., Roy, G.S., and Dutta, B.K. (1969). "Treatment and Disposal of Effluents of Modern Urea Fertiliser Factories", Seminar on Ind. Wastes, I.I.T. Kanpur, 359.

Bhattacharrya, S.H., Bhattacharrya, G.S., and Dutta, B.K., (1972). "Removal of Nitrogen from Nitrogenous Effluents", Technology, 10, 3 & 4, 321.

Birdsey, E.C., and Lynch, V.H., (1962). "Utilisation of Nitrogen Compounds by Unicellular Algae", Science, 137, 3532, 763.

Bogan, R.H., Albestson, O.E., and Pluntze, J.C., (1960). "Use of Algae in Removing Phosphorus from Sewage", J. San. Eng. Div., Proc. Am. Soc. Civil Engrs., 86, SA5, 1.

Isaac, G.S., and Tollefsen, E.L., (1975). "Luxury Uptake Phenomenon for Removal of Phosphates from Municipal Wastewater", Water Research, 9, 1, 71.

Bush, A.S., Isherwood, J.D., and Rodgi, S., (1963). "Dissolved Solids Removal from Wastewater by Algae", Trans. Am. Soc. Civil Engrs., 128, III, 84.

Borchardt, J.A., and Azad, H.S., (1968). "Biological Extraction of Nutrients", J. Water Poll. Cont. Fed., 40, 10, 1739.

Calvin, M., (1962). "Path of Carbon in Photosynthesis", Science, 135, 879.

Chakravarty, R.M., and Arceivala, S.J., (1971). "Disposal of Wastes from Fertiliser Factories", Indian J. Env. Health, 13, 1, 1.

Chang, J.P., and Morris, J.G., (1962). J. Gen. Microbiology, 28, 301.

Charbit, G.E., (1966). "Reagent for the Determination of Urea in Different Liquids", Chemical Abstracts, 65, 6, 17356.

Chari, K.S., (1976). "Indian Fertiliser Industry", Chemical Age of India, 27th Annual Issue, 139.

Chaudhuri, K.N., Meghal, S.K., and Lakshminarayana, J.S.S., (1969). "Treatment of Nitroguanidine Effluent by Algae", Seminar on Ind. Wastes, I.I.T. Kanpur, 375.

Chiaudari, G., and Vighu, G., (1974). "The N:P Ratio and Tests with Selenastrum to Predict Eutrophication in Lakes", Water Research, 8, 12, 1063.

Chrost, R.J., Owezarek, J., and Mastusick, K., (1975). "Degradation of Urea by Bacteria and Algae in Mass Algal Cultures", Acta. Microbiologica Polonica, 7, (27), 4, 231.

Clesceri, N.L., (1968). "Physical and Chemical Removal of Nutrients", Algae, Man, and the Environment, Ed., D.F. Jackson, Syracuse University Press, New York, 413.

Cousins, W.J., and Mindler, A.P., (1972). "Tertiary Treatment of Urea Ammonia Liquor", J. Water Poll. Cont. Fed., 44, 4, 607.

Culp, R.L., Wesner, G.M., and Culp, G.L., (1978). "Nitrogen Removal", Hand Book of Advanced Wastewater Treatment, 2nd Edn., Van Nostrand Reinhold Co., New York, 298.

Danforth, W.F., (1962). "Substrate Assimilation and Heterotrophy", Physiology & Biochemistry of Algae, Ed., R.A. Lewin, Academic Press, New York, 99.

Das, A.C., Khan, J.A., and Dutta, B.K., (1965). "Removal of Nitrogen from Fertiliser Factory Effluents by Biochemical Nitrification-Denitrification", Technology, 2, 1, 10.

Davis, E.A., Dedrick, J., French, C.S., Milner, H.W., Myers, J., Smith, J.H.C., and Spoehr, H.A., (1953). "Laboratory Experiments on Chlorella Culture", Algal Culture from Laboratory to Pilot Plant, Ed., J.S. Birlew, Carnegie Institution of Washington Publication, Washington D.C., 105.

Degyansky, M.E., and Sherrard, J.H., (1977). "Effects of Nitrification in the Activated Sludge Process", Water and Sewage Works, 124, 6, 94.

Dohler, R.H., (1971). "Utilisation and Uptake of Nitrogenous Compounds in Chlamydomonas reinhardtii", Dissertation Abstracts, 32, B, I, 2, 753.

Doudroff, P., and Katz, M., (1950). "Critical Review of Literature on Toxicity of Industrial Wastes and Their Components to Fish", Sewage and Industrial Wastes, 22, 11, 1432.

Englebrecht, R.S., and McKinney, R.E., (1956). "Membrane Filter Method Applied to Activated Sludge Suspended Solids Determination", Sewage and Industrial Wastes, 28, 7, 1321.

Eysler, C., (1968). "Microorganic and Microinorganic Requirements for Algae", Algae, Man and the Environment, Ed., D.F. Jackson, Syracuse University Press, New York, 27.

Farrel, T.P., Frauson, F.P., Cassel, F., and Bishop, D.F., (1972). "Nitrogen Removal by Ammonia Stripping", J. Water Poll. Cont. Fed., 44, 8, 1522.

Fleet gate, G., (1973). "Where There is Muck, There is Energy", New Scientist, 59, 853, 19.

Fogg, G.E., (1952). The Metabolism of Algae, John Wiley & Sons Inc., New York.

Francis, C.W., and Mankin, J.B., (1977). "High Nitrate Denitrification in Continuous Flow Stirred Reactors", Water Research, 11, 3, 289.

Gates, W.E., and Borchardt, J.A., (1964). "Nitrogen and Phosphorus Extraction from Domestic Wastewater Treatment Plant Effluents by Controlled Algal Cultures", J. Water Poll. Cont. Fed., 36, 4, 443.

Gerloff, G.C., (1969). "Evaluating Nutrient Supplies for the Growth of Aquatic Plants in Natural Waters", Eutrophication, National Academy of Sciences, New York, 537.

Gibson, C.E., (1971). "Nutrient Limitation", J. Water Poll. Cont. Fed., 43, 12, 2436.

Gilles, A.C., (1973). "Water, Gases and Pressure in Cell Environment", Cell Physiology, W.B. Saunders Company, Toppan Co. Ltd., Tokyo.

Gillette, L.A., Miller, D.L., and Redman, C., (1952). "Appraisal of a Chemical Waste Problem by Fish Toxicity Tests", Sewage and Industrial Wastes, 24, 11, 1397.

Golterman, H.L., (1975). Physiological Limnology, Elsevier Scientific Publishing Company, New York, 35.

Golueke, C.G., Oswald, W.J., and McGauhey, P.H., (1959). "The Biological Control of Enclosed Environments", Sewage and Industrial Wastes, 31, 10, 1125.

Golueke, C.G., Oswald, W.J., and Gee, H.K., (1967). "Effect of Nitrogen Additives on Algal Yield", J. Water Poll. Cont. Fed., 39, 5, 823.

Hattori, A., (1957). "Studies on the Metabolism of Urea and Other Nitrogenous Compounds in Chlorella ellipsodea", J. Biochem., 44, 5, 253.

Hattori, A., (1958). "Studies on the Metabolism of Urea and Other Nitrogenous Compounds in Chlorella ellipsodea", J. Biochem., 45, 1, 57.

Haug, R.T., and McCarty, P.L., (1972). "Nitrification with Submerged Filters", J. Water Poll. Cont. Fed., 44, 11, 2086.

Hendricks, D.W., and Pote, W.D., (1974). "Thermodynamic Analysis of a Primary Oxidation Pond", J. Water Poll. Cont. Fed., 46, 2, 333.

Hermann, E.R., and Gloyna, E.F., (1958). "Waste Stabilization Ponds", Sewage and Industrial Wastes, 30, 8, 963.

Hing, C.L., Obayaski, A.W., Zenz, D.R., Washington, D., and Sawyer, B.M., (1976). "Biological Nitrification of Sludge Supernatant by Rotating Discs", J. Water Poll. Cont. Fed., 48, 1, 25.

Hopson, N.E., and Sack, W.A., (1973). "Cellular Phosphorus Changes under Low Carbon Stress", J. Water Poll. Cont. Fed., 45, 1, 85.

Hoover, S.R., Jaseweiz, L., Pepinsky, J.P., and Porges, N., (1951). "Assimilation of Dairy Wastes by Activated Sludge", Sewage and Industrial Wastes, 23, 2, 167.

Hoover, S.R., and Porges, N., (1952). "Assimilation of Dairy Wastes by Activated Sludge", Sewage and Industrial wastes, 24, 3, 306.

Humenik, F.J., and Hanna, G.P., (1971). "Algal-Bacterial Symbiosis for Removal and Conservation of Wastewater Nutrients", J. Water Poll. Cont. Fed., 43, 4, 580.

Hutchinson, G.E., (1957). A Treatise on Limnology, John Wiley & Sons Inc., New York, 308 and 653.

Indian Standards Institution, (1963). "Tolerance Limits for Inland Surface Water for Fish Cultures", I.S. 2296.

Jain, A.K., Sankar, G., and Pandey, G.N., (1977). "Water Pollution and Its Abatement in Urea Plant", Chemical Age of India, 28, 6, 493.

John, U.L., (1976). Development and Evaluation of Flocculating Algal-Bacterial System for Wastewater Treatment, Ph.D. Thesis, I.I.T. Kanpur.

Kates, J.R., and Jones, R.F., (1967). "Periodic Increases in Enzyme Activity in Synchronised Cultures of Chlamydomonas reinhardtii", Biochem. et Biophy. Acta., 145, 1, 153.

Kerr, P.C., Brockway, D.L., Paris, D.F., and Barnett, J.T., (1972). "Bacterial Regulation in Aquatic Ecosystem", Proc. 26th Ind. Wastes Conf., Purdue University, Indiana, 530.

King, D.L., (1970). "The Role of Carbon in Eutrophication", J. Water Poll. Cont. Fed., 42, 12, 2035.

Krauss, R.W., (1953). "Inorganic Nutrition of Algae", Algal Culture, Carnegie Institution of Washington Publication, Washington D.C., 85.

Krauss, R.W., (1956). "Photosynthesis in Algae", Ind. and Engg. Chemistry, 48, 9, 1449.

Kuhl, A., (1967). "Phosphate Metabolism in Green Algae", Algae, Man and the Environment, Ed., D.F. Jackson, Syracuse University Press, New York, 37.

Kuhl, A., (1972). "Inorganic Phosphorus Uptake Metabolism", Physiology and Biochemistry of Algae, Ed., R.A. Lewin, Academic Press, New York, 211.

Lakshminarayana, J.S.S., Parabrahmam, M., Khan, A.N., Ratnaparkhi, D.Y. and Manuel, A.C., (1972). "Performance of Stabilisation Ponds at Bhandewadi, Nagpur", Proc. Sym. Low Cost Waste Treatment, CPHERI, Nagpur, 91.

Lewin, R.A., (1962). Physiology and Biochemistry of Algae, Academic Press, New York.

Loehr, R.C., Prakasam, T.B.S., Srinath, E.G., and Joe, Y.D., (1973). Development and Demonstration of Nutrient Removal from Farm Wastes, E. P. A., Washington D.C., 61.

Loehr, R.C., (1974). Agricultural Waste Management, Academic Press, New York, 138.

Maloney, J.E., Ludwig, H.F., Harman, J.A., and McClintock, L., (1960). "Effect of Whey on Stabilization Ponds", J. Water Poll. Cont. Fed., 32, 12, 1283.

Maloney, T.E., (1966). "Detergent Phosphorus Effect on Algae", J. Water Poll. Cont. Fed., 38, 1, 38.

Manahan, S.E., (1974). Environmental Chemistry, Willard Grant Press, Boston, Mass., 23.

Markl, H., (1977). "Carbon Dioxide Transport and Photosynthetic Productivity of a Continuous Culture of Algae", Biotech. and Bioeng., XIX, 12, 1851.

Mayer, A.M., Zuri, U., Shain, Y., and Ginzbury, H., (1964). "Problems of Design and Ecological Considerations in Mass Culture of Algae", Biotech. and Bioeng., VI, 2, 173.

McCarty, P.L., Beck, L., and St. Amant, P., (1969). "Biological Denitrification of Wastewater by Addition of Organic Chemicals", Proc. 24th Ind. Waste Conf., Purdue University, Indiana, 1271.

McCarthy, J.J., (1971). "The Role of Urea in Marine Phytoplankton Ecology", Dissertation Abstracts, 32B, II, 4, 2310.

McFeters, G.A., Stuart, S.A., and Obson, S.B., (1978). "Growth of Heterotrophic Bacteria and Algal Extra Cellular Products in Oligotrophic Waters", Appl. and Env. Microbiology, 35, 2, 383.

McGriff, E., Jr., (1970). Nutrient Removal by Activated Algae, Ph.D. Thesis, University of Kansas, 35.

McKee, F.J., (1950). "Milk Waste Treatment by Aeration", Sewage and Industrial Wastes, 22, 8, 1041.

McKinney, R.E., (1941). "Chlorophyll Extraction", Biological Chemistry, 140, 315.

Mercer, B.M., Ames, L.L., Tonbill, C.J., Vanslyke, W.J., and Dean, R.B., (1970). "Ammonia Removal from Secondary Effluents by Selective Ion Exchange", J. Water Poll. Cont. Fed., 42, 2, Pt. 2, R 95.

Metcalf & Eddy Inc., (1974). Wastewater Engineering, Tata McGraw Hill Publishing Co., New Delhi, 401.

Millen, J.A., Long, D.A., and Cherniak, M.J., (1975). "Characterisation and Treatment Feasibility of Milking Center Waste", Proc. 30th Ind. Waste Conf., Purdue University, Indiana, 861.

Mohan Rao, G.J., and Subrahmanyam, P.V.R., (1973). "Aspects of Water Pollution in Fertiliser Industry", Indian J. Env. Health, 15, 4, 271.

Myers, J., (1953). "Growth Characteristics of Algae in Relation to the Problems of Mass Culture", Algal Culture, Ed., J.S. Birlew, Carnegie Institute of Washington Publication, Washington D.C., 37. ,

Natarajan, K.V., (1970). "Toxicity of Ammonia to Marine Diatoms", J. Water Poll. Cont. Fed., 42, 5, II, R184.

Nemerow, N.L., (1971). "Treatment of Dairy Wastes", Liquid Waste of Industry, Addison-Wesley Publishing Company, Reading, Mass., 316.

Oswald, W.J., Gotaas, H.B., Ludwig, H.F., and Lynch, V., (1951). "Algae Symbiosis in Oxidation Ponds", Sewage and Industrial Wastes, 23, 11, 1337.

Oswald, W.J., Gotaas, H.B., Ludwig, H.F., and Lynch, V., (1953). "Algae Symbiosis in Oxidation Ponds", Sewage and Industrial Wastes, 25, 1, 26.

Oswald, W.J., Gotaas, H.B., Golueke, C.G., and Kellen, W.R., (1957). "Algae in Waste Treatment", Sewage and Industrial Wastes, 29, 4, 437.

Oswald, W.J., (1960). "Light Conversion Efficiency of Algae Grown in Sewage", J. San. Eng. Div., Proc. Am. Soc. Civil Engrs., SA4, 2558, 71.

Oswald, W.J., (1963). "Light Conversion Efficiency of Algae Grown in Sewage", Transactions Am. Soc. Civil Engrs., 128, 3, 47.

Pearson, E.A., (1968). "Kinetics of Biological Treatment", Advances in Water Quality Improvement, Ed., E.F. Gloyana and W.W. Eckenfelder, Jr., University of Texas Press, Austin, 381.

Penman, H.L., (1970). "The Water Cycle", Scientific American, 223, 3, 98.

Pillai, V.S., (1976). "Liquid Effluents from Ammonia-Urea Plants", Pollution in Fertiliser Industry in India, FAI, Group Discussion, New Delhi, 16.

Pipes, W.O., (1961). "Basic Biology of Stabilisation Ponds", Water and Sewage Works, 108, 4, 131.

Pipes, W.O., (1961). "Algae Growth Rate", Water and Sewage Works, 108, 5, 176.

Quirk, T.P., and Hellman, J., (1972). "Activated Sludge and Trickling Filtration Treatment of Whey Effluents", J. Water Poll. Cont. Fed., 44, 12, 2277.

Rabinowitch, E.I., (1951). Photosynthesis, Vol. II, Pt. I, "Photosynthesis and Related Processes", Interscience Publishing Co., New York, 28.

Rabinowitch, E.I., (1956). "Kinetics of Photosynthesis", Photosynthesis, Vol. II, Pt. II, Interscience Publishing Co., New York, 31.

Rabinowitch, E.I., and Govindjee, (1973). "The Light and the Dark Stage", Photosynthesis, Wiley Eastern Private Ltd., New Delhi, 56.

Ramalingiah, (1974). "Studies on Coagulant Combination in Concentrating Algae", M.Tech. Thesis, I.I.T. Kanpur.

Rao, A.V.S.P., and Ramprasad, G., (1971). "A Study on Air-Stripping of Ammonia", Indian J. Env. Health, 13, 2, 105.

Reeves, T.G., (1972). "Nitrogen Removal — A Literature Review", J. Water Poll. Cont. Fed., 44, 10, 1895.

Roesler, J.F., (1971). "Simulation of Ammonia-Stripping from Wastewater", J. San. Eng. Div., Proc. Am. Soc. Civil Engrs., 97, SA3, 269.

Round, F.E., (1966). The Biology of the Algae, Edward Arnold (Publishers) Ltd., London, 171.

Ryther, J.H., and Dunstan, W.M., (1971). "Nitrogen, Phosphorus and Eutrophication in Coastal Marine Environment", Science, 171, 1008.

Sanders, W.M., (1972). "Nutrients", River Ecology and Man, Academic Press, New York, 389.

Sawyer, C.N., (1952). "Some New Aspects of Phosphates in Relation to Lake Eutrophication", Sewage and Industrial Wastes, 24, 6, 768.

Sawyer, C.N., (1962). "Causes, Effects and Control of Aquatic Growths", J. Water Poll. Cont. Fed., 34, 3, 279.

Sawyer, C.N., and McCarty, P.L., (1967). Chemistry for Sanitary Engineers, 2nd Edn., McGraw-Hill Book Co., New York.

Sculze, K.L., (1966). "Biological Recovery of Wastewater", J. Water Poll. Cont. Fed., 38, 12, 1944.

Shen, Y.S., and Chen, C.S., (1965). Advanced Water Pollution Research Proceedings and International Conference, Tokyo, Pergamon Press, London.

Shah, D.B., and Coulman, G.A., (1978). "Kinetics of Nitrification and Denitrification Reactors", Biotech. and Bioeng., XX, 1, 43.

Shapario, J., and Ribeiro, R., (1965). "Removal of Phosphates from Sewage Effluent", J. Water Poll. Cont. Fed., 37, 1034.

Siddiqui, R.H., Ratnaparkhi, D.Y., and Agarwal, S.C. (1971). "Nitrification Treatment of Nitrogenous Fertiliser Wastewater", Indian J. Env. Health, 13, 2, 128.

Siddiqui, R.H., and Sehgal, T.R., (1972). "Some Observations on Operation of a Facultative Stabilisation Pond", Proc. Syn. Low Cost Waste Treatment, CPHERI, Nagpur, 85.

Smit, A.C.M., and Kassenbrood, P.J.C., (1976). "Treatment of Urea Plant Effluents", Industrial Wastes, 22, 5, 44.

Sorokin, G., (1960). "Kinetic Studies of Temperature Effects on the Cellular Level", Biochemi et Biophy. Acta., 38.

Southgate, B.A., (1952). "Pollution on Streams", Sewage and Industrial Wastes, 24, 8, 1045.

St. Amant, P.P.S., and McCarty, P.L., (1969). "Treatment of High Nitrate Waters", J. Am. Water Works Assn., 61, 12, 659.

Standard Methods for the Examination of Water and Wastewater, (1965). 12th Edn., American Public Health Association, Inc., New York.

Stanier, R.Y., Doudoroff, M., and Adelberg, E.A. (1974). General Microbiology, 3rd Edn., Macmillan India, New Delhi, 715.

Stensel, H.P., Loehr, R.C., and Lawrence, A.W., (1973). "Biological Kinetics of Suspended Growth Denitrification", J. Water Poll. Cont. Fed., 45, 2, 249.

Sherrad, J.H., and Schroeder, E.D., (1975). "Stoichiometry of Industrial Biological Wastewater Treatment", Proc. 30th Ind. Waste Conf., Purdue University, Indiana, 14.

Stephensen, R.L., (1977). "A Micro-Kjeldahl Technique for Nitrogen Determination in Water, Wastewater and Sludge", J. Water Poll. Cont. Fed., 49, 12, 2499.

Stumm, W., and Morgan, J.J., (1970). "Interaction between Organisms and Abiotic Environment", Aquatic Chemistry, Wiley Interscience, New York, 429.

Syrett, P.J., (1972). "Nitrogen Assimilation", Physiology and Biochemistry of Algae, Ed., R.A. Lewin, Academic Press, New York, 171.

Ussig, H.H., (1952). "Some Aspects of the Application of Tracers in Permeability Studies", Advances in Enzymology, 13, 21.

Vacker, D., Connel, C.H., and Wells, W.N., (1967). "Phosphate Removal through Municipal Wastewater Treatment at San Antonis, Texas", J. Water Poll. Cont. Fed., 39, 4, 750.

Verstraete, W., Vanstaen, H., and Voets, J.P., (1977). "Adaptation to Nitrification of Activated Sludge System Treating Highly Nitrogenous Water", J. Water Poll. Cont. Fed., 49, 7, 1604.

Warren, K.S., (1962). "Ammonia Toxicity and pH", Nature, 195, 4836, 47.

Water Quality Criteria, Federal Water Pollution Control Administration, (1968), 68.

Weber, W.J., Jr., (1972). Physicochemical Process for Water Quality Control, Wiley Interscience, New York, 350.

Williams, S.K., and Hudson, R.C., (1977). "Transport of Urea at Low Concentration in Chlamydomonas reinhardtii", J. Bacteriology, 130, 1, 266.

Willie, J., (1939). Canadian Public Health Journal, 28, 128.

Wilt, V., and Borchardt, J.A., (1960). "The Removal of Nitrogen and Phosphorus from Sewage Effluent Through Use of Algal Culture", J. Biochem. and Microbiol. Tech. and Eng., II, 2, 187.

Wood, K.E., (1968). "Photosynthesis of Cladaphora Under Unnatural Conditions", Algae, Man and the Environment, Ed., D.F. Jackson, Syracuse University Press, New York.

Zajic, J.E., and Chu, Y.S., (1970). "Heterotrophic Growth of Algae", Properties and Products of Algae, Ed., J.E. Zajic, Plenum Press, New York.

APPENDIX A.1 - VALUES OF F AT DIFFERENT pH AND TEMPERATURES

Temperature (°C)	pH								
	7.0	7.5	8.0	8.5	9.0	9.5	10.0	10.5	11.0
10	0.002	0.006	0.020	0.061	0.170	0.393	0.672	0.866	0.953
15	0.003	0.009	0.028	0.082	0.221	0.473	0.739	0.900	0.966
20	0.004	0.012	0.037	0.110	0.280	0.552	0.796	0.925	0.975
25	0.005	0.017	0.052	0.148	0.354	0.634	0.846	0.945	0.982
30	0.008	0.025	0.076	0.207	0.452	0.723	0.892	0.963	0.988
35	0.014	0.043	0.125	0.312	0.589	0.819	0.935	0.978	0.993

APPENDIX A.2 - VALUES OF F AND CORRESPONDING VALUES OF (1-F)/F

F	(1-F)/F	F	(1-F)/F	F	(1-F)/F
0.01	99.0000	0.30	2.3333	0.70	0.4286
0.02	49.0000	0.32	2.1250	0.72	0.3889
0.03	32.3333	0.34	1.9412	0.74	0.3514
0.04	24.0000	0.36	1.7778	0.75	0.3333
0.05	19.0000	0.38	1.6316	0.76	0.3158
0.06	15.6667	0.40	1.5000	0.78	0.2821
0.07	13.2857	0.42	1.3810	0.80	0.2500
0.08	11.5000	0.44	1.2727	0.82	0.2195
0.09	10.1111	0.46	1.1739	0.84	0.1905
0.10	9.0000	0.48	1.0833	0.86	0.1628
0.12	7.3333	0.50	1.0000	0.88	0.1364
0.14	6.1429	0.52	0.9231	0.90	0.1111
0.16	5.2500	0.54	0.8519	0.92	0.0869
0.18	4.5556	0.56	0.7857	0.94	0.0638
0.20	4.0000	0.58	0.7241	0.96	0.0416
0.22	3.5455	0.60	0.6667	0.98	0.0204
0.24	3.1667	0.62	0.6129	0.99	0.0101
0.25	3.0000	0.64	0.5625	1.00	0
0.26	2.8462	0.66	0.5152		
0.28	2.5714	0.68	0.4706		

APPENDIX B - CALIBRATION CURVES

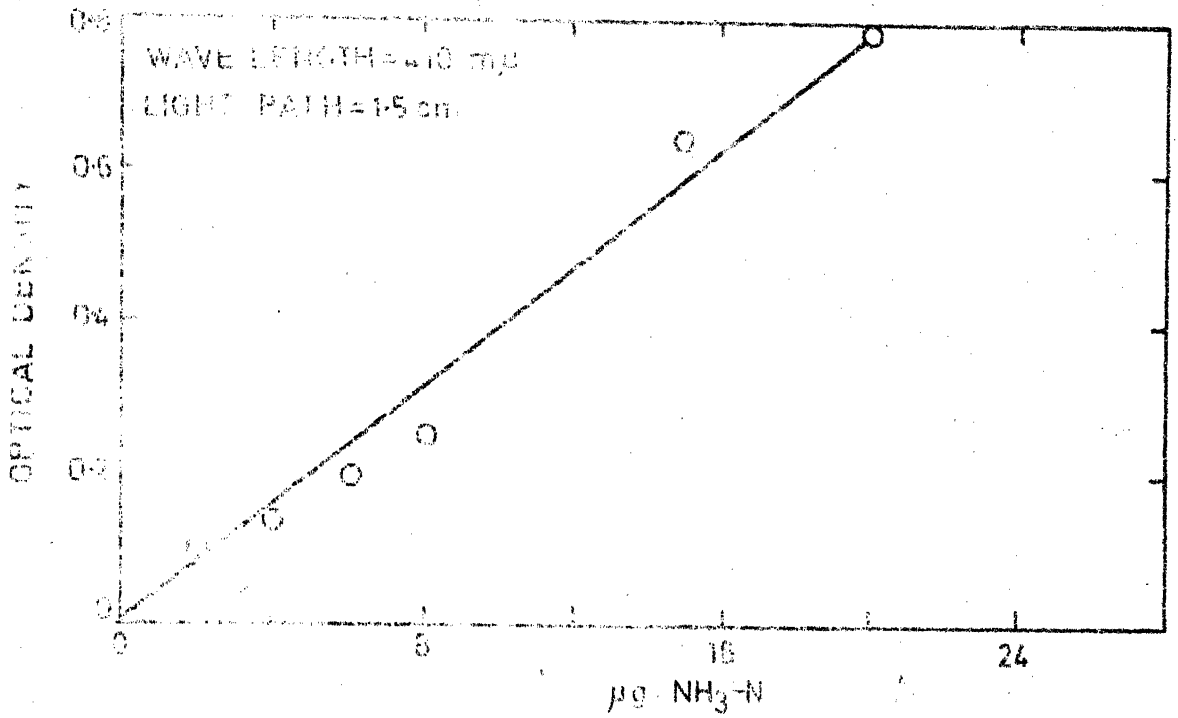


FIG. A1 STANDARD CURVE FOR AMMONIA NITROGEN

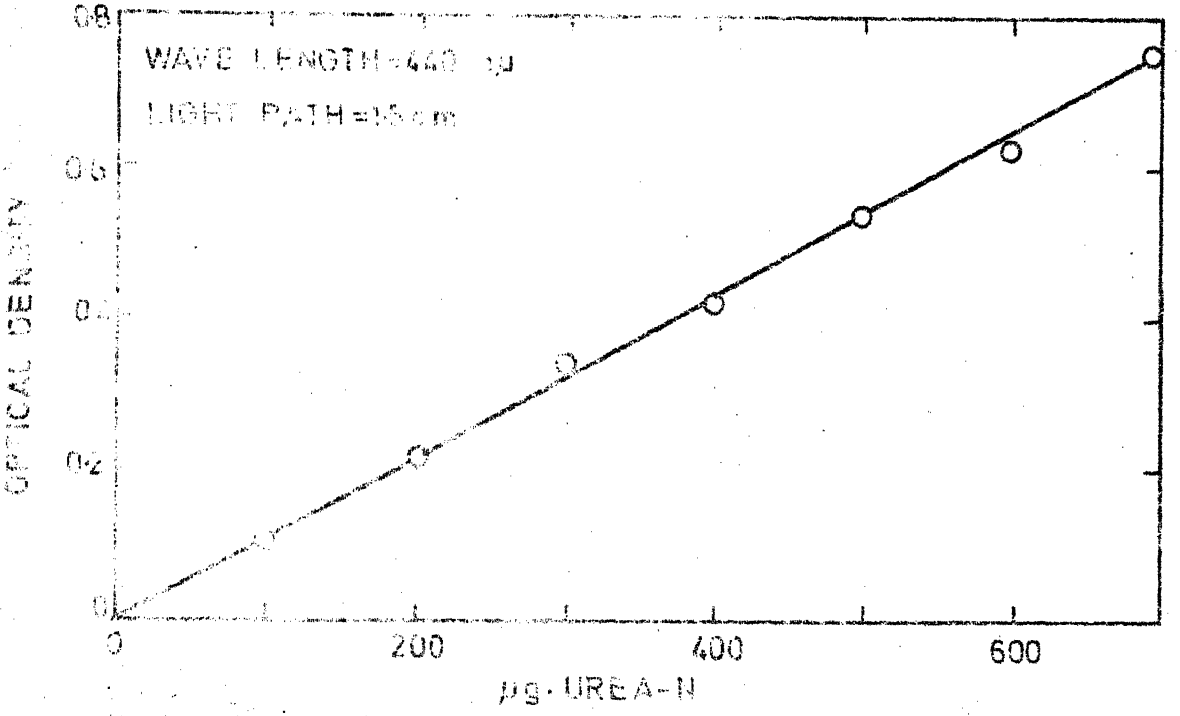


FIG. A2 STANDARD CURVE FOR UREA NITROGEN

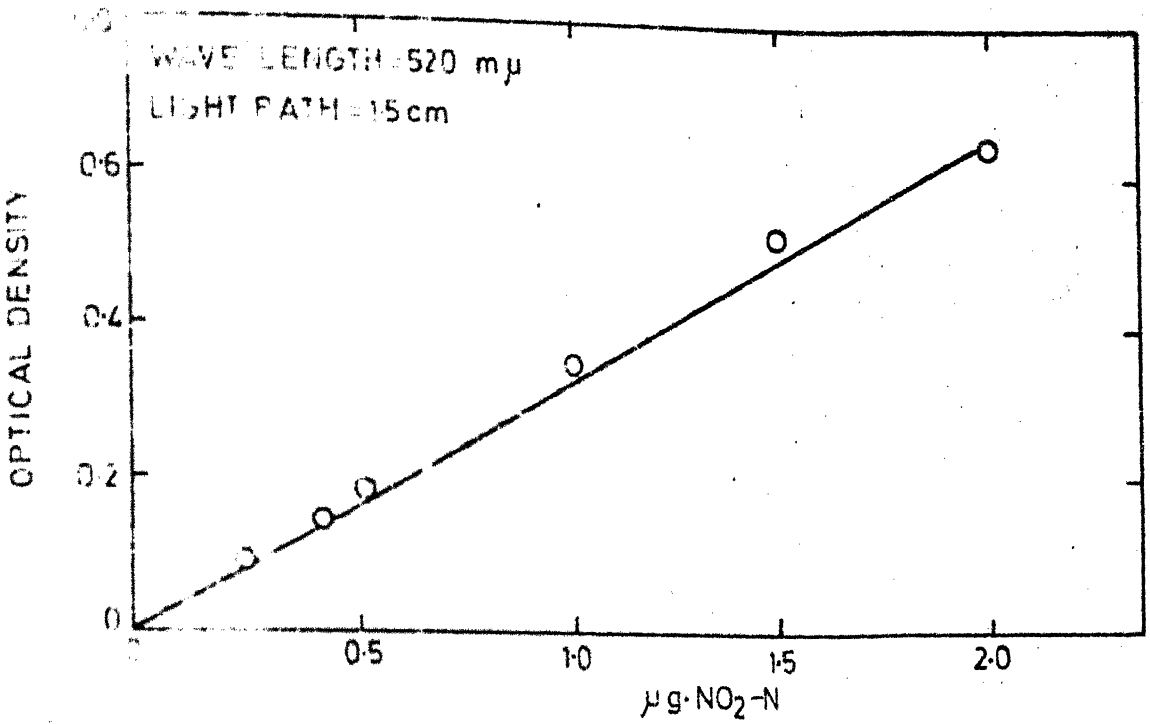


FIG.A3 STANDARD CURVE FOR NITRITE NITROGEN

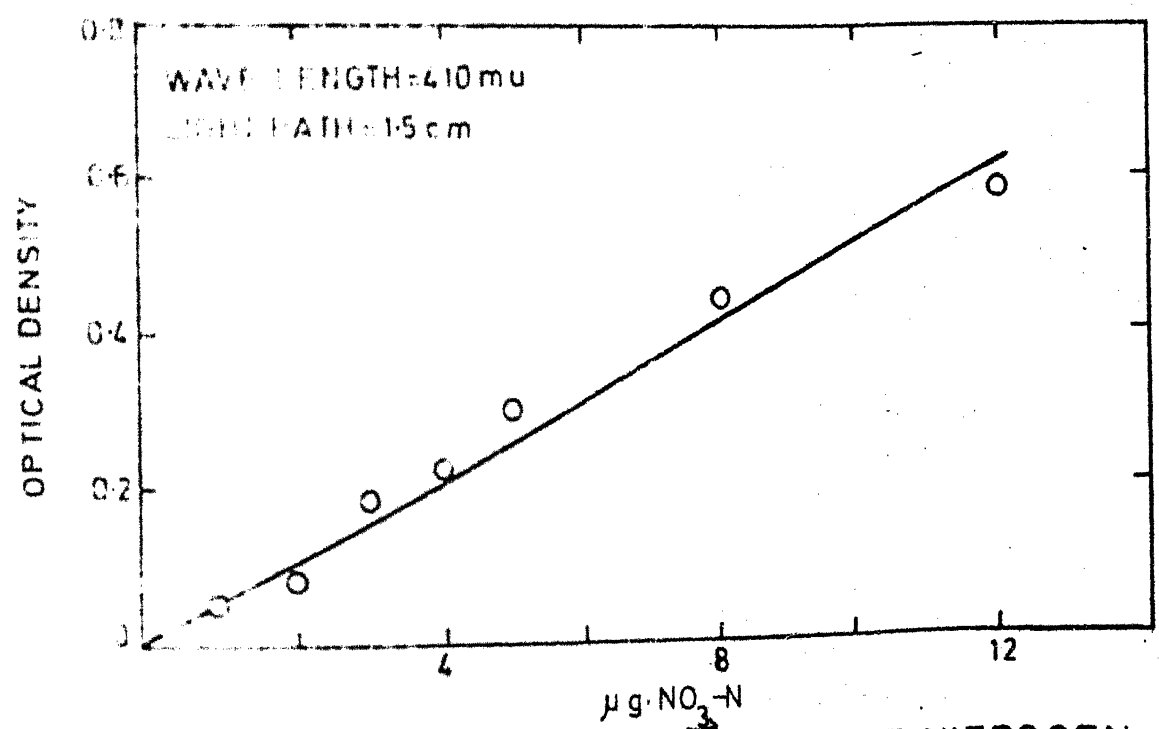


FIG.A4 STANDARD CURVE FOR NITRATE NITROGEN

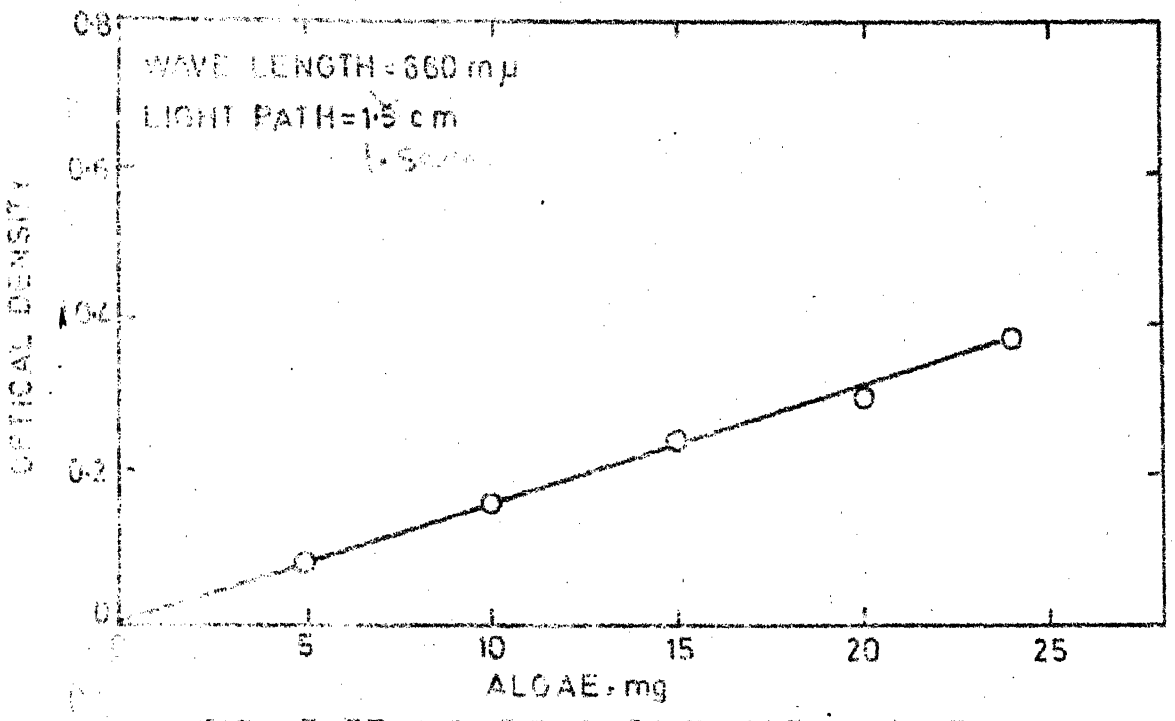


FIG.A5 STANDARD CURVE FOR ALGAE

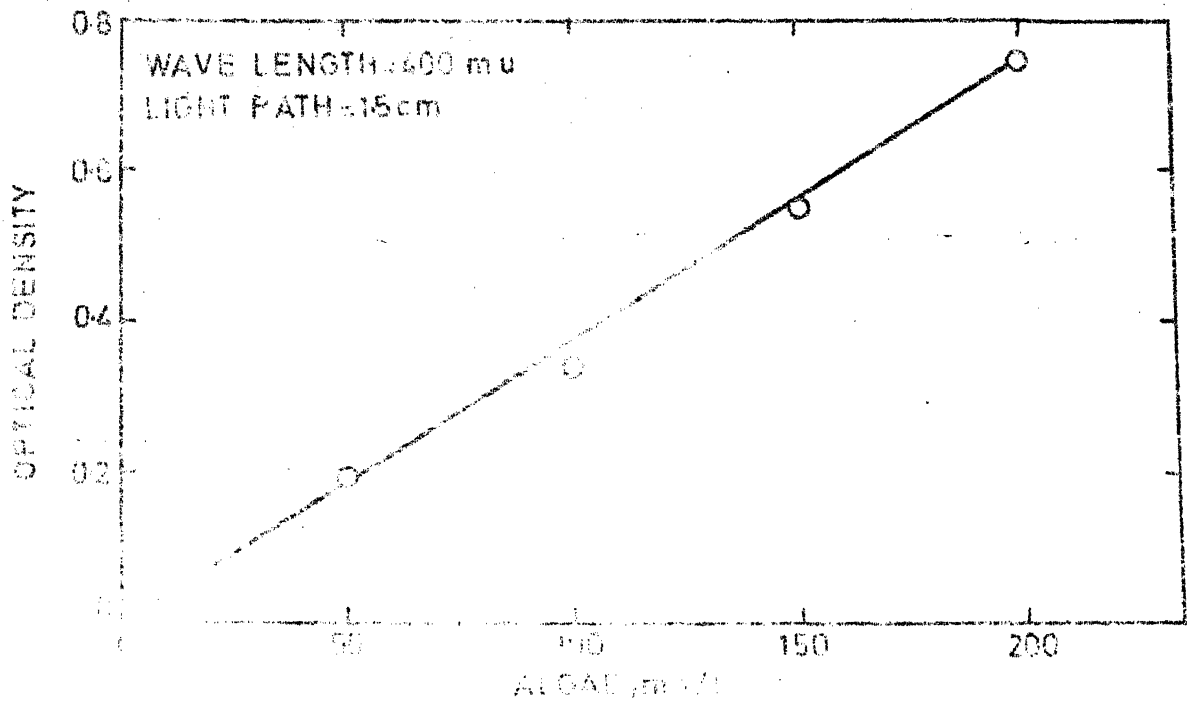


FIG.A6 STANDARD CURVE FOR EFFLUENT

APPENDIX C.1 – EXPERIMENTAL RESULTS FROM PHASE 1 - PART 1*

Days	Biomass conc. mg/l	Algae conc. mg/l	Algae bacteria	Influent				Effluent				Total N removal %	COD removal %
				NH_3-N mg/l	COD mg/l	pH	Alkalnity mg/l	NH_3-N mg/l	NO_2^--N mg/l	NO_3^--N mg/l	COD mg/l		
				Reactor 1 - C:N:P ratio 30:10:1									
0	1400	850	1.54	100	770	7.9	500						
2	1600					7.5	120	25	0	0	40	75	95
4	2000	1200	1.50	150	950	7.6	500	25	3	2	40	75	95
6	2100					7.3	130	25	3	2	0	70	100
8	2400	1400	1.40	200	1470	7.6	510	50	5	3	0	65	100
10	2800					7.0	120	25	12	3	110	78	93
12	4000	2200	1.22	250	1840	7.8	500	55	6	4	110	68	94
14	4600					6.7	110	50	6	4	110	70	94
16	5400	3200	1.45			6.7	110	50	6	4	110	74	94
18	6600	3700	1.27			6.7	110	50	6	4	110	74	94
				Reactor 2 - C:N:P ratio 30:10:1									
0	1400	850	1.54	300	2300	7.9	510						
2	1400					7.8	130	125	0	1	120	58	95
4	1300	800	1.22	500	3650	7.8	510	75	0	1	120	75	95
6	1900					7.2	120	150	3	2	160	69	96
8	2700	1400	1.08	675	5340	7.8	510	125	3	2	150	74	96
10	4600					6.7	130	150	3	3	150	77	97
12	5800	3300	1.32	975	7360	7.9	500	150	6	3	240	76	96
14	7400					6.7	110	275	6	4	180	71	98
16	7000	4000	1.33			6.6	110	300	12	4	180	68	98
18	6600	3700	1.27			6.6	110	300	12	4	180	68	98

Continued...

Appendix C.1 continued

Days	Biomass conc. mg/l	Algae conc. mg/l	Algae bacteria	Influent				Effluent				Total N removal %	COD removal %
				NH_3-N mg/l	COD mg/l	pH	Alkalinity mg/l	NH_3-N mg/l	NO_2^--N mg/l	NO_3^--N mg/l	COD mg/l		
				Reactor 3 - C:N:P ratio 30:10:1									
0	1400	850	1.54	400	3070	7.9	510						
2	1100					7.4	140	125	0	2	120	69	96
4	1400	870	1.64	575	4080	7.8	510	125	6	2	160	67	95
6	2800					6.5	110	125	3	2	160	77	96
8	3800	2200	1.38	750	4600	7.9	510	150	6	3	240	72	94
10	4600					6.3	110	250	5	4	240	67	95
12	5200	3000	1.36	975	7540	7.9	510	200	5	4	280	72	94
14	7600					6.2	120	275	5	5	280	71	96
16	6900	3900	1.30			6.2	110	275	5	6	280	71	96
18	6700	3800	1.31			6.2	110	275	5	8	280	70	96
				Reactor 4 - C:N:P ratio 30:10:1									
0	1400	850	1.54	500	3650	7.9	520						
2	1200					7.2	140	150	0	2	150	70	96
4	1400	800	1.33	650	5380	7.9	520	150	0	2	150	70	96
6	2700					6.9	110	225	2	2	190	65	96
8	3700	2000	1.18	950	7730	7.8	510	200	2	3	240	68	95
10	4800					6.3	110	300	3	3	240	68	97
12	5600	3700	1.95	1000	7450	7.9	510	250	5	4	370	73	95
14	7800					6.2	110	325	5	5	330	74	96
16	7400	4400	1.46			6.2	110	300	6	6	280	67	96
18	6900	4000	1.38			6.2	110	275	6	6	280	69	96

* Temperature 33-41°C.

APPENDIX C.2 - EXPERIMENTAL RESULTS FROM PHASE 1 - PART 2*

Days	Biomass conc. mg/l	Algae conc. mg/l	Algae / Bacteria	Influent				Effluent						Total N removal %	COD removal %
				NH_3-N mg/l	COD mg/l	pH	Alkalinity mg/l	NH_3-N mg/l	NO_2^--N mg/l	NO_3^--N mg/l	COD mg/l	pH	Alkalinity mg/l		
				Reactor 1 - C:N:P ratio 30:10:1											
0	7000	3600	1.10	975	7490	8.3	4.80								
2	10000	4900	0.96					225	15	5	307	7.1	120	75	96.0
4	7200							200	18	5	263	7.2	120	77	97.5
6	7500	3600	0.92					175	22	5	225	7.0	120	80	97.0
8	7600							175	18	5	188	6.5	120	80	97.5
10	6600	3100	0.88					150	18	5	112	6.4	120	82	98.0
				Reactor 2 - C:N:P ratio 25:10:1											
0	5600	3100	1.24	950	6340	8.1	480								
2	8200	4500	1.22					250	14	4	345	7.2	110	73	94.0
4	5600							175	16	5	307	6.6	110	81	95.0
6	6200	3200	1.06					150	22	5	265	6.6	100	82	96.0
8	7100							150	28	5	225	6.5	100	81	96.5
10	5800	3300	1.32					150	24	5	188	6.5	100	82	97.0
				Reactor 3 - C:N:P ratio 20:10:1											
0	5600	3400	1.54	950	5180	8.3	480								
2	6400	3800	1.46					275	18	4	270	7.6	110	70	95.0
4	5400							200	20	5	230	6.6	110	77	96.0
6	7500	4600	1.58					175	24	5	225	6.5	110	79	96.0
8	6200							175	24	5	119	6.5	110	79	96.5
10	6000	4000	1.50					150	24	5	188	6.5	110	81	96.5
				Reactor 4 - C:N:P ratio 15:10:1											
0	5000	3200	1.77	950	3650	8.2	480								
2	6000	3800	1.73					250	20	4	192	7.2	100	72	95.0
4	5000							200	18	4	192	7.3	100	77	95.0
6	4200	2700	1.80					175	26	4	154	6.8	110	82	96.0
8	4400							175	28	4	154	6.6	110	79	96.0
10	4100	2800	2.15					175	22	4	112	6.6	110	79	97.0

* Temperature 29-34°C.

APPENDIX C.3 - EXPERIMENTAL RESULTS FROM PHASE 1 - PART 3

Days	Biomass conc. mg/l	Algae conc. mg/l	Algae / Bacteria	Influent				Effluent						Total N removal %	COD removal %
				NH_3-N mg/l	COD mg/l	pH	Alkalinity mg/l	NH_3-N mg/l	NO_2^--N mg/l	NO_3^--N mg/l	COD mg/l	pH	Alkalinity mg/l		
				Reactor 1 - C:N:P ratio 20:10:1 with no carbon dioxide											
0	6200	3400	1.34	950	5290	8.0	460								
2	6000	3600	1.50					400	18	3	215	6.9	180	56	92
4	5400	3400	1.70					350	20	4	275	6.1	120	61	92
6	6000	3500	1.40					325	20	8	257	6.6	120	62	93
8	6200	3600	1.40					325	22	8	257	6.3	120	62	93
10	6000	3500	1.40					400	20	8	331	6.0	120	50	91
				Reactor 2 - C:N:P ratio 15:10:1 with no carbon dioxide											
0	5200	3200	1.70	975	3530	7.9	460								
2	5600	3400	1.54					400	18	3	313	6.9	80	57	94
4	5400	3400	1.70					350	20	9	275	6.1	120	61	95
6	4800	2600	1.20					350	20	12	258	6.6	120	61	95
8	5000	2800	1.27					350	22	14	258	6.3	120	61	95
10	4000	2300	1.35					425	20	12	368	6.0	120	54	93
				Reactor 3 - C:N:P ratio 20:10:1 with carbon dioxide											
0	5600	3600	1.89	950	5100	7.9	460								
2	6200	4000	1.82					350	20	2	274	7.0	120	61	92
4	6000	4000	2.00					325	28	2	274	6.5	120	63	92
6	6400	4200	1.92					325	26	3	257	6.5	100	63	93
8	7000	4500	1.80					325	25	6	231	6.2	120	61	91
10	6000	4000	2.00					375	26	8	331	6.2	120	57	91
				Reactor 4 - C:N:P ratio 15:10:1 with carbon dioxide											
0	5000	3200	1.77	950	3530	7.9	460								
2	6000	4000	2.00					325	22	3	352	6.8	100	63	93
4	6400	4000	1.67					325	25	4	275	6.4	100	63	95
6	6200	4000	1.80					350	23	4	257	6.5	120	60	95
8	6600	4600	2.30					300	24	6	220	6.2	120	65	96
10	6400	4600	2.55					375	22	6	368	6.4	120	56	93

* Temperature 24-31°C.

APPENDIX C.4 - EXPERIMENTAL RESULTS FROM PHASE 1 - PART 4*

Days	Biomass conc. mg/l	Algae conc. mg/l	Algae / Bacteria	Influent NH_3-N mg/l	Influent COD mg/l	Influent pH	Influent Alkalinity mg/l	Effluent NH_3-N mg/l	Effluent NO_2^--N mg/l	Effluent NO_3^--N mg/l	Effluent COD mg/l	Effluent pH	Effluent Alkalinity mg/l	Total N removal %	COD removal %
				Reactor 1 - C:N:P ratio 14:10:1											
0	4000	2500	1.7	1050	3460	7.9	510								
2	6100	3800	1.7					600	27	8	77	6.4	100	35	98
4	6400	4400	2.2					575	30	8	77	6.0	80	37	98
6	6000	4200	2.3					575	22	9	77	6.0	120	37	98
8	6700	4400	1.9					550	23	10	77	6.0	80	41	98
10	6700	4400	1.9					550	21	12	38	5.6	60	41	99
				Reactor 2 - C:N:P ratio 6:10:1											
0	4000	2900	2.6	950	1550	7.8	510								
2	4200	2900	2.3					625	30	9	77	6.3	120	30	95
4	4400	3000	2.1					600	28	9	77	6.1	100	33	95
6	3600	2800	3.5					650	27	11	115	6.0	140	28	93
8	4400	3000	2.1					550	31	12	77	6.5	60	38	95
10	4300	2900	2.1					600	30	12	77	6.9	80	33	95
				Reactor 3 - C:N:P ratio 3:10:1											
0	3800	2800	2.8	950	770	7.8	500								
2	3600	2800	3.5					650	31	9		6.1	160	27	100
4	3900	2800	2.5					625	33	12	37	6.1	100	30	90
6	3000	2300	3.3					650	22	12	115	6.0	140	27	85
8	3300	2500	3.1					625	26	12	77	6.2	80	31	90
10	3500	2600	2.9					625	31	12	38	6.9	80	30	95
				Reactor 4 - Sewage with supplemented organic carbon (C:N:P - 3:10:1)											
0	3800	2400	1.7	950	810	7.8	500								
2	3600	2400	2.0					650	30	11		6.4	160	28	100
4	3600	2600	2.6					625	34	11	77	6.1	140	30	91
6	3600	2500	2.7					650	32	12	77	6.0	100	27	91
8	3000	2500	5.0					625	29	12	77	6.3	80	30	91
10	3000	2600	4.5					625	28	12		7.0	80	30	100

* Temperature 12-19°C.

APPENDIX C.5 - EXPERIMENTAL RESULTS FROM PHASE 1 - PART 5*

Days	Biomass conc. mg/l	Algae conc. mg/l	Algae Bacteria	Influent				Effluent						Total N removal %	COD removal %
				NH_3-N mg/l	COD mg/l	pH	Alkalinity mg/l	NH_3-N mg/l	NO_2^--N mg/l	NO_3^--N mg/l	COD mg/l	pH	Alkalinity mg/l		
Reactor 1 - C:N:P ratio 6:10:1; no pH adjustment															
0	4700	3000	1.8	975	1550	7.8	500								
2	5100	3800	2.9					600	10	1	37	6.6	120	37	98
4	6800	4200	1.6					500	14	5	111	6.4	120	47	93
6	5400	4200	3.5					500	15	8	111	6.2	80	46	93
8	4200	3000	2.5					500	21	14	148	6.2	60	45	90
10	4000	3000	3.0					500	16	22	185	6.3	60	45	88
Reactor 2 - C:N:P ratio 6:10:1; pH 8.0-8.3 with Na_2CO_3															
0	4700	3000	1.8	975	1510	7.8	500								
2	4500	3000	2.0					550	18		185	6.8	200	42	88
4	5000	3400	2.1					525	21	7	111	6.9	200	44	93
6	4600	3000	1.9					400	20	14	148	6.9	160	55	90
8	3600	2400	2.0					450	25	24	111	6.7	160	49	93
10	3800	2600	2.2					400	22	34	185	7.0	160	53	88
Reactor 3 - C:N:P ratio 6:10:1; pH 8.0-8.3 with $Ca(OH)_2$															
0	4700	3000	1.8	975	1580	7.8	500								
2	5000	3800	3.2					525	21		74	6.9	200	45	95
4	4200	2800	2.0					500	26	9	111	6.6	200	45	93
6	3600	2600	2.6					450	23	19	111	7.7	180	55	93
8	3600	2400	2.0					400	24	22	148	7.5	180	54	91
10	4000	2600	1.9					400	20	34	148	7.0	180	54	91

* Temperature 8-18°C.

APPENDIX C.6 - EXPERIMENTAL RESULTS FROM PHASE I - PART C

Days	Biomass conc. mg/l	Algae conc. mg/l	Algae / Bacteria	Influent				Effluent						Total N removal %	COD removal %
				NH_3-N mg/l	COD mg/l	pH	Alkalinity mg/l	NH_3-N mg/l	NO_2^--N mg/l	NO_3^--N mg/l	COD mg/l	pH	Alkalinity mg/l		
				Reactor 1 - C:N:P ratio 6:10:1; activated sludge system											
0	5000			1000	1505	7.5	470								
1	8700							675	29		219	7.4		29	85
2	5200							875	31	1	384	7.4	100	10	75
4	5000							525	44	2	268	7.4		43	82
6	4000							525	35	2	192	7.5	80	44	87
8	4000							600	31	6	77	7.0		37	95
10	4000							600	20	10		7.3	70	37	100
				Reactor 2 - C:N:P ratio 6:10:1; algal system											
0	3500			1050	1570	8.0	470								
1	3200							675	25	2	537	7.4		33	66
2	3400							700	22	3	192	6.9	70	31	88
4	2600							525	20	3	154	7.2		48	90
6	2500							500	20	3	154	7.2	70	51	90
8	2400							500	20	3		7.0		51	100
10	2400							500	22	3		7.1	70	51	100
				Reactor 3 - C:N:P ratio 6:10:1; algal-bacterial system											
0	4200	2400	1.33	975	1570	7.9	470								
1	4400							625	32	1		7.2		33	100
2	4800	3200	1.50					675	30	5	192	6.9	70	28	88
4	5000	3500	2.30					475	30	8	154	6.7		48	90
6	4000							425	28	8	159	6.7	70	53	90
8	4400	3300	3.00					375	25	10		7.0		58	100
10	4600	3500	3.20					350	24	10		7.0	70	61	100
				Reactor 4 - C:N:P ratio 6:10:1; simple aeration system											
0				975	1440	8.0	470								
1								975			1440	8.0	375		
2								950	20	5	1374	7.5	300	5	5
4								800	21	2	346	7.1		16	76
6								700	20	2	154	7.2	140	26	89
8								600	20	2		7.2		36	100
10								600	20	6		6.9	70	36	100

* Temperature 5-18°C.

APPENDIX C.7 - EXPERIMENTAL RESULTS FROM PHASE 1 - PART 7*

Days	Biomass conc. mg/l	Algae conc. mg/l	Algae/Bacteria	Influent NH_3-N mg/l	Influent Urea N mg/l	Influent COD mg/l	pH	Alkalinity mg/l	Effluent NH_3-N mg/l	Effluent NO_2^--N mg/l	Effluent NO_3^--N mg/l	Effluent Urea N mg/l	Effluent COD mg/l	Sludge COD mg/l	Sludge N mg/l	Organic N mg/l	Total N removal %	COD removal %
							Reactor 1 - C:N:P ratio 5:10:1											
0	4000	2200	1.3	650	290	1270	8.2	500						3200	425			
2	5400	2800	1.1				6.6	350	300	50	8	100	536	3800	500		51	58
4	4800	2500	1.1				6.7	230	300	45	19	50	308	3400	550	100	46	74
6	3800	2600	2.2				6.7	140	325	48	40	20	515	3100	550		44	60
8	3300	2800	5.6				7.0	100	250	35	50		628	3800	500	125	51	51
10	3500	2900	4.8				6.9	20	300	45	45		698	3600	500		45	45
							Reactor 2 - C:N:P ratio 7:10:1											
0	4000	2200	1.3	650	310	1800	8.2	520						3100	475			
2	5600	2800	1.0				6.8	350	325	38	7	100	308	3700	500		51	83
4	4000	2400	1.7				6.3	220	300	48	10	60	308	3000	550	50	51	83
6	3800	2500	1.9				6.2	120	300	45	22		308	3300	600		54	83
8	3400	2900	5.8				6.2	110	300	40	32		294	3800	500	100	51	84
10	3100	2600	5.2				6.2	40	500	40	24		698	3700	500		51	62
							Reactor 3 - C:N:P ratio 9:10:1											
0	4200	2600	1.6	675	310	2310	8.1	480						3700	400			
2	5600	2900	1.1				6.2	320	300	38	6	60	77	4200	500		59	97
4	4800	2700	1.3				6.4	200	275	45	10	40	472	3700	525		57	82
6	3500	2800	2.5				6.1	120	250	45	24		626	4200	550	50	57	73
8	4000	3200	4.0				6.1	100	300	50	32		515	4000	500		51	78
10	3700	3100	5.1				6.0	40	350	48	24		698	3700	400	125	43	70
							Reactor 4 - C:N:P ratio 10:10:1											
0	4500	2600	1.4	675	300	2680	8.1	480						4100	425			
2	5800	3100	1.2				6.2	340	350	35	1	40		4800	550		57	100
4	5000	3000	1.5				6.8	190	275	40	10		691	4000	600	50	62	74
6	3900	2000	3.3				6.1	120	250	45	35		538	4100	525	100	55	80
8	4800	3400	2.4				6.1	100	250	35	40		404	4200	500		55	85
10	4000	3200	4.0				6.0	40	300	35	35		588	3900	500	100	52	78

* Temperature 15-28°C.

APPENDIX D. AMMONIA LOST DURING AERATION

D.1. Calculation of Desorption Coefficient, K_d:

The formula given by Loehr et.al., (1973) is used in the calculation. The formula is:

$$\log_e \frac{C_1}{C_2} = K_d \cdot F \cdot (t_2 - t_1) \qquad (D.1)$$

where C_1 = Concentration of total ammonia at time t_1

C_2 = Concentration of total ammonia at time t_2

K_d = Desorption coefficient which is dependent on the rate of air flow, volume of air and mass transfer coefficient

F = Proportion of free ammonia to total ammonia.

Values obtained during the experimental studies are tabulated as follows:

Table D1 - Ammonia N Concentration and pH and Time of Aeration at Temperature 15-28°C

No.	Initial NH_3N Concentration mg/l	Final NH_3N Concentration mg/l	Time in Days	pH	
				Initial	Final
1	975	950	2	8.1	7.5
2	1000	975	2	8.0	7.8
3	950	950	2	8.0	7.5

Choosing a set of values from this table, we have

$$C_1 = 975 \text{ mg/l}$$

$$C_2 = 950 \text{ mg/l}$$

$$t_1 = 0$$

$$t_2 = 2 \text{ days}$$

Taking average values of temperature and pH, we have

$$\text{Temperature} = 22^{\circ}C$$

$$\text{pH} = 7.8$$

For these values of temperature and pH, value of F is taken from Appendix A.1 and are reproduced as in Table D2.

Table D2 - Values of F at Different Temperature and pH

	Temperature °C	pH	
		7.5	8.0
Values of F	20	0.012	0.037
	25	0.017	0.052

Interpretating these values, we get:

$$F \text{ at } 20^{\circ}C \text{ at pH } 7.8 = 0.012 + \frac{0.025 \times 0.3}{0.5} = 0.027$$

$$F \text{ at } 25^{\circ}C \text{ at pH } 7.8 = 0.017 + \frac{0.035 \times 0.3}{0.5} = 0.038$$

$$F \text{ at } 22^{\circ}C \text{ at pH } 7.8 = 0.027 + \frac{2}{5} \times 0.038 = 0.042$$

Substituting this value of F = 0.042 in Equation (D.1), we get the value of K_d as follows:

$$\log_e \frac{C_1}{C_2} = K_d \cdot F \cdot (t_2 - t_1)$$

or $$\log_e \frac{975}{950} = K_d \cdot 0.042 \cdot 2$$

or $$K_d = \frac{0.0255}{0.042 \times 2} = 0.3035$$

This value of K_d has been used to find out the quantity of ammonia N stripped by air.

D.2. Calculation of Ammonia Stripped in Phase 1 - Part 7

The values are reproduced from Appendix C.7:

C_1 = 650 mg/l; Temperature = 25°C; pH = 8.1

At the end of 2 days pH dropped below 7.0. From Appendix A.1 at temperature 25°C and pH 8.1 value of F can be obtained as 0.052. This calculation holds good for Reactors 1 and 2 in Phase 1 - Part 7. Substituting this values in Equation (D.1), we get

$$\log_e \frac{650}{C_2} = 0.3035 \cdot 0.052 \cdot 2 = 0.0312$$

or $$C_2 = \frac{650}{1.032} = 630 \text{ mg/l}$$

Ammonia N lost during aeration in 2 days in Reactors 1 and 2

$$= (C_1 - C_2) = (650 - 630) = 20 \text{ mg/l}$$

For Reactors 3 and 4 the value of $C_1 = 675$ mg/l

Proceeding as in the earlier case, we get:

$$\log_e \frac{675}{C_2} = 0.3035 \cdot 0.052 \cdot 2 = 0.0312$$

or $$C_2 = \frac{675}{1.032} = 654$$

Ammonia N lost during aeration in 2 days in Reactors 3 and 4

$$= (C_1 - C_2) = (675 - 654) = 21 \text{ mg/l}$$

APPENDIX E.1 - EXPERIMENTAL RESULTS FROM PHASE 2 - PART 1*

No.	Influent Characteristics									Detention time days
	Biomass conc. mg/l	Algae conc. mg/l	Algae / Bacteria	NH_3-N mg/l	Urea N mg/l	Other N mg/l	COD mg/l	pH	Alkalinity mg/l	
Reactor 1 - C:N:P ratio 10:10:1 - no pH control										
1	5000	3100	1.6	650	300		2690	7.6	440	2
2	5400	3200	1.5	675	300		2730	8.0	440	2
3	5200	3400	1.9	650	320		2650	8.1	450	2
4	5100	3200	1.7	675	310		2650	7.9	430	2
5	4900	3500	2.5	650	320		2690	8.2	450	2
Reactor 2 - Influent = Effluent of Reactor 1 + Organic carbon										
1	4900	2900	1.5	500	90	30	1920	6.8	120	2
2	5000	3000	1.5	525	60	56	1960	6.7	200	2
3	5100	3200	1.7	500	60	85	2000	6.7	220	2
4	4900	3200	1.9	500	90	100	2000	6.7	180	2
5	4900	3100	1.7	475	90	70	1920	6.7	160	2
Reactor 3 - Influent = Effluent of Reactor 2 + Organic carbon										
1	5000	3100	1.6	375		35	1540	6.0	80	2
2	4800	3000	1.9	375		65	1540	6.1	120	2
3	4900	3100	1.7	375		85	1470	6.2	80	2
4	4900	3200	1.9	375		95	1510	5.9	140	2
5	5100	3200	1.7	350		70	1470	5.8	120	2

* Temperature 28-37°C.

Continued...

ppendix E.1 continued

o.	Effluent Characteristics										Total N removal %	COD removal %
	Biomass conc. mg/l	Algae conc. mg/l	Algae/Bacteria	NH_3-N mg/l	Urea N mg/l	NO_2^--N mg/l	NO_3^--N mg/l	COD mg/l	pH	Alkalinity mg/l		
	Reactor 1 - C:N:P ratio 10:10:1 - no pH control											
1	5900	3200	1.9	500	90	30		150	6.8	120	35	94
2	5300	4000	3.1	525	60	50	6	190	6.7	200	34	93
3	5400	4000	2.8	500	60	60	25	120	6.8	220	33	95
4	5700	3900	2.2	500	90	60	40	280	6.7	180	30	89
5	5400	4000	2.8	475	90	40	30	250	6.7	160	35	91
	Reactor 2 - Influent = Effluent of Reactor 1 + Organic carbon											
1	5200	3800	2.7	375		30	5	380	6.0	80	34	80
2	5700	4100	2.6	375		40	25	350	6.1	120	31	82
3	5300	3600	2.1	375		45	40	260	6.2	80	29	87
4	5400	3800	2.4	375		45	50	260	5.9	140	32	87
5	5500	3800	2.2	350		30	40	300	5.8	120	34	84
	Reactor 3 - Influent = Effluent of Reactor 2 + Organic carbon											
1	5300	3600	2.1	300		30	20	150	5.8	60	15	90
2	5100	3300	1.8	360		30	40	150	5.9	80	3	90
3	5600	3500	1.8	300		40	40	110	5.9	80	17	93
4	5400	3400	1.7	300		30	50	220	5.8	80	19	85
5	5600	3500	1.7	310		40	30	110	5.8	80	10	93

APPENDIX E.2 - EXPERIMENTAL RESULTS FROM PHASE 2 - PART 2*

o.	Influent Characteristics									Detention time days
	Biomass conc. mg/l	Algae conc. mg/l	Algae / Bacteria	NH_3-N mg/l	Urea N mg/l	Other N mg/l	COD mg/l	pH	Alkalinity mg/l	
	Reactor 1 - C:N:P ratio 10:10:1 - no pH control Biomass concentration = 5000 mg/l									
1	4800	3000	1.7	675	300		2730	7.9	460	2
2	5000	3100	1.6	650	300		2920	7.6	460	2
3	4900	2900	1.5	675	300		2670	8.1	480	2
4	5000	2900	1.4	650	320		2650	8.3	460	2
5	5200	3100	1.5	675	310		2650	7.9	450	2
	Reactor 2 - Influent = Effluent of Reactor 1 + Organic carbon Biomass concentration = 1400 - 1600 mg/l									
1	1800	1200	2.0	475	60	20	2000	6.2	120	2
2	1500	900	1.5	475	30	70	2000	6.4	180	2
3	1600	1000	1.6	475	40	90	1920	6.3	220	2
4	1700	1000	1.4	450	50	60	1920	6.3	180	2
5	1600	1100	2.2	450	40	75	1920	6.3	160	2
	Reactor 3 - Influent = Effluent of Reactor 2 + Organic carbon Biomass concentration = 1400 - 1600 mg/l									
1	1400	900	1.8	350		55	1500	5.6	100	2
2	1600	900	1.3	350		80	1540	5.9	80	2
3	1500	1000	2.0	325		75	1470	5.9	60	2
4	1600	1100	2.2	350		75	1510	5.7	80	2
5	1400	1000	2.5	325		70	1470	5.7	80	2

* Temperature 29-38°C.

Continued...

ppendix E.2 continued

).	Effluent Characteristics										Total N removal	COD removal
	Biomass conc. mg/l	Algae conc. mg/l	Algae Bacteria	NH_3-N mg/l	Urea N mg/l	NO_2^--N mg/l	NO_3^--N mg/l	COD mg/l	pH	Alkalinity mg/l	%	%
	Reactor 1 - C:N:P ratio 10:10:1 - no pH control Biomass concentration = 5000 mg/l											
1	5800	3600	1.6	475	60	20		190	6.2	120	43	93
2	5300	3500	1.9	475	30	45	25	160	6.4	180	40	95
3	5400	3500	1.8	475	40	55	35	120	6.3	220	38	95
4	5700	3600	1.9	450	50	25	35	250	6.3	180	42	91
5	5400	3400	1.7	450	40	35	40	280	6.3	160	43	89
	Reactor 2 - Influent = Effluent of Reactor 1 + Organic carbon Biomass concentration = 1400 - 1600 mg/l											
1	3400	2100	1.6	350		35	20	120	5.6	100	28	94
2	3100	2000	1.8	350		50	30		5.9	80	25	100
3	3300	2200	2.0	325		40	35	80	5.9	60	34	96
4	3100	2400	3.4	350		35	40	40	5.7	80	24	98
5	3400	2400	2.4	325		30	40	40	5.9	80	30	98
	Reactor 3 - Influent = Effluent of Reactor 2 + Organic carbon Biomass concentration = 1400 - 1600 mg/l											
1	3300	2100	1.8	225		30	35		5.4	60	29	100
2	3400	2300	2.1	225		25	30	40	5.5	40	35	97
3	3600	2500	2.3	200		30	40		5.5	60	35	100
4	3300	2000	1.6	200		30	45	40	5.5	40	35	97
5	3100	2100	2.1	200		30	45	40	5.5	40	30	97

APPENDIX E.3 - EXPERIMENTAL RESULTS FROM PHASE 2 - PART 3*

No.	Influent Characteristics									Detention time days
	Biomass conc. mg/l	Algae conc. mg/l	Algae / Bacteria	NH_3-N mg/l	Urea N mg/l	Other N mg/l	COD mg/l	pH	Alkalinity mg/l	
	Reactor 1 - C:N:P ratio 10:10:1 - pH at 8.0 - 8.3 Biomass concentration = 5000 mg/l									
1	5200	3200	1.6	650	290		2650	8.2	660	2
2	5300	3200	1.5	675	310		2460	8.3	650	2
3	5100	3300	1.8	650	310		2650	8.0	670	2
4	4900	3100	1.7	675	300		2650	8.1	670	2
5	5200	3300	1.7	650	320		2650	8.2	670	2
	Reactor 2 - Influent = Effluent of Reactor 1 + Organic carbon Biomass concentration = 1400 - 1600 mg/l; pH at 8.0 - 8.3									
1	1600	1000	1.6	400	50	40	1920	8.1	680	2
2	1500	1000	2.0	375	30	80	1750	8.3	670	2
3	1700	1200	2.4	350	30	110	1840	8.2	680	2
4	1700	1100	1.8	350	20	100	1670	8.2	650	2
5	1600	1100	2.2	350	30	90	1840	8.3	660	2
	Reactor 3 - Influent = Effluent of Reactor 2 + Organic carbon Biomass concentration = 1400 - 1600 mg/l; pH at 8.0 - 8.3									
1	1500	900	1.5	125		45	960	8.3	660	2
2	1400	900	1.8	200		75	960	8.2	630	2
3	1700	1100	1.8	175		75	1010	8.3	640	2
4	1600	1000	1.6	125		75	840	8.3	660	2
5	1500	1000	2.0	150		60	870	8.1	650	2

* Temperature 29-40°C.

Continued...

ppendix E.3 continued

o.	Effluent Characteristics										Total N removal %	COD removal %
	Biomass conc. mg/l	Algae conc. mg/l	Algae/Bacteria	NH_3-N mg/l	Urea N mg/l	NO_2^--N mg/l	NO_3^--N mg/l	COD mg/l	pH	Alkalinity mg/l		
	Reactor 1 - C:N:P ratio 10:10:1 - pH at 8.0 - 8.3 Biomass concentration = 5000 mg/l											
1	5400	3500	1.8	400	50	30	10	190	6.8	280	48	93
2	5400	3700	2.1	375	30	50	30	350	6.9	260	49	86
3	5300	3500	1.9	350	30	60	50	240	7.0	260	49	91
4	5600	3600	1.8	350	20	50	50	180	6.8	200	52	93
5	5400	3400	1.7	350	30	40	50	240	7.1	240	52	91
	Reactor 2 - Influent = Effluent of Reactor 1 + Organic carbon Biomass concentration = 1400 - 1600 mg/l; pH at 8.0 - 8.3											
1	3300	2200	2.0	125		20	25	100	6.1	180	65	95
2	3100	2000	1.8	200		35	30		6.0	220	45	100
3	3100	2000	1.8	175		35	40		5.9	240	49	100
4	3400	2400	2.4	125		30	45	40	6.2	180	58	98
5	3100	2100	2.1	150		30	30		6.3	180	55	100
	Reactor 3 - Influent = Effluent of Reactor 2 + Organic carbon Biomass concentration = 1400 - 1600 mg/l; pH at 8.0 - 8.3											
1	3200	2000	1.7	25		25	30	100	5.8	210	53	90
2	3200	2000	1.7	75		25	25		5.9	220	53	100
3	3300	2400	2.1	50		35	25		6.2	240	56	100
4	3000	2200	2.7	25		30	35		6.1	260	55	100
5	3100	2200	2.4	25		25	30	40	6.0	180	62	96

VITA

Name:	K. Raghavan Nambiar
Date of Birth:	November 12, 1936

Academic

E.S.L.C. (1949)	Government of Madras, obtained First Class with Distinction; First Rank holder in the school.
S.S.L.C. (1952)	Board of Secondary Education, Madras, obtained High First Class; First Rank holder in the school.
Intermediate (1954)	University of Madras, obtained First Class with Distinction; Winner of Science Association Prize being Topper in the subjects.
B.E. (Hons.) (1958)	University of Madras, obtained First Class with Distinction and a high rank in the university.
M.Sc. (Engg.) (1963)	University of Madras, obtained First Class with Distinction and a high rank in the university.
Ph.D. (1978)	Indian Institute of Technology, Kanpur, credited 8 subjects and obtained a C.P.I. of 9.75 out of a maximum of 10.0.

Professional

Junior Engineer (1958-61)	Public Works Departments of Madras and Kerala States.
Lecturer in Civil Engg. (1961-69)	Government Engineering College, Trichur, Kerala, presently under University of Cochin.
Assistant Professor in Civil Engineering (1969-)	Government Engineering College, Trichur, Kerala, presently under University of Cochin. Guided 2 students for M.Sc.(Engg.) under University of Calicut. Published 1 research paper in the Journal of the Institution of Engineers (India).